

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 February 2007 (08.02.2007)

PCT

(10) International Publication Number
WO 2007/014924 A1

(51) International Patent Classification:

C07K 5/078 (2006.01) A61K 31/41 (2006.01)
A61K 38/05 (2006.01) A61P 31/14 (2006.01)
C07D 403/04 (2006.01)

(21) International Application Number:

PCT/EP2006/064818

(22) International Filing Date: 28 July 2006 (28.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

05107072.0 29 July 2005 (29.07.2005) EP

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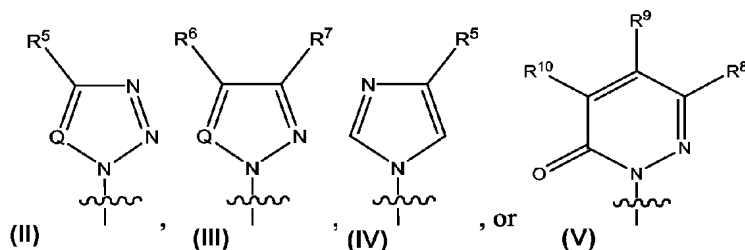
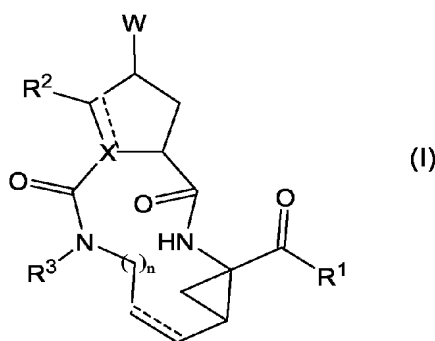
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: MACROCYCLIC INHIBITORS OF HEPATITIS C VIRUS



(57) Abstract: Inhibitors of HCV replication of formula (I), wherein W is a heterocycle of formula (II), (III), (IV), or (V); and the remaining variables are as defined in the specification.

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- with international search report

MACROCYCLIC INHIBITORS OF HEPATITIS C VIRUS

The present invention is concerned with macrocyclic compounds having inhibitory activity on the replication of the hepatitis C virus (HCV). It further concerns
5 compositions comprising these compounds as active ingredients as well as processes for preparing these compounds and compositions.

Hepatitis C virus is the leading cause of chronic liver disease worldwide and has become a focus of considerable medical research. HCV is a member of the
10 *Flaviviridae* family of viruses in the *hepacivirus* genus, and is closely related to the *flavivirus* genus, which includes a number of viruses implicated in human disease, such as dengue virus and yellow fever virus, and to the animal *pestivirus* family, which includes bovine viral diarrhea virus (BVDV). HCV is a positive-sense, single-stranded
15 RNA virus, with a genome of around 9,600 bases. The genome comprises both 5' and 3' untranslated regions which adopt RNA secondary structures, and a central open reading frame that encodes a single polyprotein of around 3,010-3,030 amino acids. The polyprotein encodes ten gene products which are generated from the precursor polyprotein by an orchestrated series of co- and posttranslational endoproteolytic cleavages mediated by both host and viral proteases. The viral structural proteins
20 include the core nucleocapsid protein, and two envelope glycoproteins E1 and E2. The non-structural (NS) proteins encode some essential viral enzymatic functions (helicase, polymerase, protease), as well as proteins of unknown function. Replication of the viral genome is mediated by an RNA-dependent RNA polymerase, encoded by non-structural protein 5b (NS5B). In addition to the polymerase, the viral helicase and
25 protease functions, both encoded in the bifunctional NS3 protein, have been shown to be essential for replication of HCV RNA. In addition to the NS3 serine protease, HCV also encodes a metalloproteinase in the NS2 region.

Following the initial acute infection, a majority of infected individuals develop chronic
30 hepatitis because HCV replicates preferentially in hepatocytes but is not directly cytopathic. In particular, the lack of a vigorous T-lymphocyte response and the high propensity of the virus to mutate appear to promote a high rate of chronic infection. Chronic hepatitis can progress to liver fibrosis leading to cirrhosis, end-stage liver disease, and HCC (hepatocellular carcinoma), making it the leading cause of liver
35 transplantations.

There are 6 major HCV genotypes and more than 50 subtypes, which are differently distributed geographically. HCV type 1 is the predominant genotype in Europe and the

US. The extensive genetic heterogeneity of HCV has important diagnostic and clinical implications, perhaps explaining difficulties in vaccine development and the lack of response to therapy.

- 5 Transmission of HCV can occur through contact with contaminated blood or blood products, for example following blood transfusion or intravenous drug use. The introduction of diagnostic tests used in blood screening has led to a downward trend in post-transfusion HCV incidence. However, given the slow progression to the end-stage liver disease, the existing infections will continue to present a serious medical and
10 economic burden for decades.

Current HCV therapies are based on (pegylated) interferon-alpha (IFN- α) in combination with ribavirin. This combination therapy yields a sustained virologic response in more than 40% of patients infected by genotype 1 viruses and about 80% of
15 those infected by genotypes 2 and 3. Beside the limited efficacy on HCV type 1, this combination therapy has significant side effects and is poorly tolerated in many patients. Major side effects include influenza-like symptoms, hematologic abnormalities, and neuropsychiatric symptoms. Hence there is a need for more effective, convenient and better tolerated treatments.

20 Recently, two peptidomimetic HCV protease inhibitors have gained attention as clinical candidates, namely BILN-2061 disclosed in WO00/59929 and VX-950 disclosed in WO03/87092. A number of similar HCV protease inhibitors have also been disclosed in the academic and patent literature. It has already become apparent
25 that the sustained administration of BILN-2061 or VX-950 selects HCV mutants which are resistant to the respective drug, so called drug escape mutants. These drug escape mutants have characteristic mutations in the HCV protease genome, notably D168V, D168A and/or A156S. Accordingly, additional drugs with different resistance patterns are required to provide failing patients with treatment options, and combination therapy
30 with multiple drugs is likely to be the norm in the future, even for first line treatment.

Experience with HIV drugs, and HIV protease inhibitors in particular, has further emphasized that sub-optimal pharmacokinetics and complex dosage regimes quickly result in inadvertent compliance failures. This in turn means that the 24 hour trough
35 concentration (minimum plasma concentration) for the respective drugs in an HIV regime frequently falls below the IC₉₀ or ED₉₀ threshold for large parts of the day. It is considered that a 24 hour trough level of at least the IC₅₀, and more realistically, the IC₉₀ or ED₉₀, is essential to slow down the development of drug escape mutants.

Achieving the necessary pharmacokinetics and drug metabolism to allow such trough levels provides a stringent challenge to drug design. The strong peptidomimetic nature of prior art HCV protease inhibitors, with multiple peptide bonds poses pharmacokinetic hurdles to effective dosage regimes.

5

There is a need for HCV inhibitors which may overcome the disadvantages of current HCV therapy such as side effects, limited efficacy, the emerging of resistance, and compliance failures.

10 WO04/072243 relates to macrocyclic hepatitis C serine protease inhibitors; pharmaceutical compositions comprising the aforementioned compounds for administration to a subject suffering from HCV infection; and methods of treating an HCV infection in a subject by administering a pharmaceutical composition comprising the said compounds.

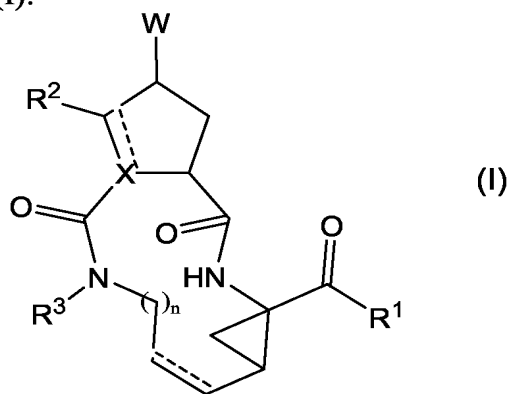
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The present invention concerns HCV inhibitors which are superior in one or more of the following pharmacological related properties, i.e. potency, decreased cytotoxicity, improved pharmacokinetics, improved resistance profile, acceptable dosage and pill burden.

In addition, the compounds of the present invention have relatively low molecular weight and are easy to synthesize, starting from starting materials that are commercially available or readily available through art-known synthesis procedures.

25

The present invention concerns inhibitors of HCV replication, which can be represented by formula (I):



30

and the *N*-oxides, salts, and stereoisomers thereof, wherein

each dashed line (represented by - - - -) represents an optional double bond;

X is N, CH and where **X** bears a double bond it is C;

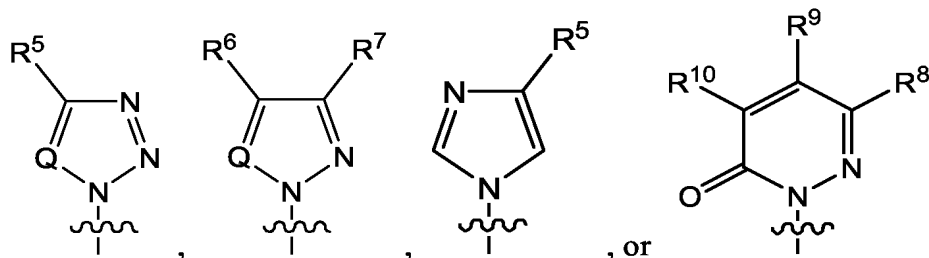
R¹ is -OR¹¹, -NH-SO₂R¹²;

5 **R**² is hydrogen, and where **X** is C or CH, **R**² may also be C₁₋₆alkyl;

R³ is hydrogen, C₁₋₆alkyl, C₁₋₆alkoxyC₁₋₆alkyl, or C₃₋₇cycloalkyl;

n is 3, 4, 5, or 6;

W is a heterocycle of formula



10 **Q** is N or CR⁴;

R⁴ is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl;

R⁵ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl
optionally substituted with C₁₋₆alkyl; or pyridyl;

15 one of **R**⁶, **R**⁷ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl; while the other of **R**⁶, **R**⁷
is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl;

one of **R**⁸, **R**⁹, **R**¹⁰ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
20 thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl; while the other two of
R⁸, **R**⁹, **R**¹⁰ independently are hydrogen; phenyl optionally substituted with
C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or
pyridyl;

R¹¹ is hydrogen; aryl; Het; C₃₋₇cycloalkyl optionally substituted with C₁₋₆alkyl; or
25 C₁₋₆alkyl optionally substituted with C₃₋₇cycloalkyl, aryl or with Het;

R¹² is aryl; Het; C₃₋₇cycloalkyl optionally substituted with C₁₋₆alkyl; or C₁₋₆alkyl
optionally substituted with C₃₋₇cycloalkyl, aryl or with Het;

each **aryl** as a group or part of a group is phenyl, optionally substituted with one, two
or three substituents selected from halo, hydroxy, nitro, cyano, carboxyl, C₁₋₆alkyl,
30 C₁₋₆alkoxy, C₁₋₆alkoxyC₁₋₆alkyl, C₁₋₆alkylcarbonyl, amino, mono- or
diC₁₋₆alkylamino, azido, mercapto, polyhaloC₁₋₆alkyl, polyhaloC₁₋₆alkoxy,
C₃₋₇cycloalkyl, pyrrolidinyl, piperidinyl, piperazinyl, 4-C₁₋₆alkyl-piperazinyl,

4-C₁₋₆alkylcarbonyl-piperazinyl, morpholinyl; wherein the morpholinyl and piperidinyl groups may be optionally substituted with one or with two C₁₋₆alkyl radicals; and

each **Het** as a group or part of a group is a 5 or 6 membered saturated, partially
5 unsaturated or completely unsaturated heterocyclic ring containing 1 to 4
heteroatoms each independently selected from nitrogen, oxygen and sulfur, and
being optionally substituted with one, two or three substituents each independently
selected from the group consisting of halo, hydroxy, nitro, cyano, carboxyl,
C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkoxyC₁₋₆alkyl, C₁₋₆alkylcarbonyl, amino, mono- or
10 diC₁₋₆alkylamino, azido, mercapto, polyhaloC₁₋₆alkyl, polyhaloC₁₋₆alkoxy,
C₃₋₇cycloalkyl, pyrrolidinyl, piperidinyl, piperazinyl, 4-C₁₋₆alkyl-piperazinyl,
4-C₁₋₆alkylcarbonyl-piperazinyl, morpholinyl; wherein the morpholinyl and
piperidinyl groups may be optionally substituted with one or with two C₁₋₆alkyl
radicals.

15 The invention further relates to methods for the preparation of the compounds of
formula (I), the *N*-oxides, addition salts, quaternary amines, metal complexes, and
stereochemically isomeric forms thereof, their intermediates, and the use of the
intermediates in the preparation of the compounds of formula (I).

20 The invention relates to the compounds of formula (I) *per se*, the *N*-oxides, addition
salts, quaternary amines, metal complexes, and stereochemically isomeric forms
thereof, for use as a medicament. The invention further relates to pharmaceutical
compositions comprising the aforementioned compounds for administration to a subject
25 suffering from HCV infection. The pharmaceutical compositions may comprise
combinations of the aforementioned compounds with other anti-HCV agents.

30 The invention also relates to the use of a compound of formula (I), or a *N*-oxide,
addition salt, quaternary amine, metal complex, or stereochemically isomeric forms
thereof, for the manufacture of a medicament for inhibiting HCV replication. Or the
invention relates to a method of inhibiting HCV replication in a warm-blooded animal
said method comprising the administration of an effective amount of a compound of
formula (I), or a *N*-oxide, addition salt, quaternary amine, metal complex, or
stereochemically isomeric forms thereof.

35 As used in the foregoing and hereinafter, the following definitions apply unless
otherwise noted.

The term halo is generic to fluoro, chloro, bromo and iodo.

5 The term “polyhaloC₁₋₆alkyl” as a group or part of a group, e.g. in polyhaloC₁₋₆alkoxy, is defined as mono- or polyhalo substituted C₁₋₆alkyl, in particular C₁₋₆alkyl substituted with up to one, two, three, four, five, six, or more halo atoms, such as methyl or ethyl with one or more fluoro atoms, for example, difluoromethyl, trifluoromethyl, trifluoroethyl. Preferred is trifluoromethyl. Also included are perfluoroC₁₋₆alkyl groups, which are C₁₋₆alkyl groups wherein all hydrogen atoms are replaced by fluoro
10 atoms, e.g. pentafluoroethyl. In case more than one halogen atom is attached to an alkyl group within the definition of polyhaloC₁₋₆alkyl, the halogen atoms may be the same or different.

15 As used herein “C₁₋₄alkyl” as a group or part of a group defines straight or branched chain saturated hydrocarbon radicals having from 1 to 4 carbon atoms such as for example methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 2-methyl-1-propyl; “C₁₋₆alkyl” encompasses C₁₋₄alkyl radicals and the higher homologues thereof having 5 or 6 carbon atoms such as, for example, 1-pentyl, 2-pentyl, 3-pentyl, 1-hexyl, 2-hexyl, 2-methyl-1-butyl, 2-methyl-1-pentyl, 2-ethyl-1-butyl, 3-methyl-2-pentyl, and
20 the like. Of interest amongst C₁₋₆alkyl is C₁₋₄alkyl.

The term “C₂₋₆alkenyl” as a group or part of a group defines straight and branched chained hydrocarbon radicals having saturated carbon-carbon bonds and at least one double bond, and having from 2 to 6 carbon atoms, such as, for example, ethenyl (or
25 vinyl), 1-propenyl, 2-propenyl (or allyl), 1-butenyl, 2-butenyl, 3-butenyl, 2-methyl-2-propenyl, 2-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 2-methyl-2-butenyl, 2-methyl-2-pentenyl and the like. Of interest amongst C₂₋₆alkenyl is C₂₋₄alkenyl.

30 The term “C₂₋₆alkynyl” as a group or part of a group defines straight and branched chained hydrocarbon radicals having saturated carbon-carbon bonds and at least one triple bond, and having from 2 to 6 carbon atoms, such as, for example, ethynyl, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 3-butyne, 2-pentyne, 3-pentyne, 2-hexynyl, 3-hexynyl and the like. Of interest amongst C₂₋₆alkynyl is C₂₋₄alkynyl.

35 C₃₋₇cycloalkyl is generic to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

C₁₋₆alkanediyl defines bivalent straight and branched chain saturated hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, methylene, ethylene, 1,3-propanediyl, 1,4-butanediyl, 1,2-propanediyl, 2,3-butanediyl, 1,5-pentanediy, 1,6-hexanediy and the like. Of interest amongst C₁₋₆alkanediyl is C₁₋₄alkanediyl.

5

C₁₋₆alkoxy means C₁₋₆alkyloxy wherein C₁₋₆alkyl is as defined above.

As used herein before, the term (=O) or oxo forms a carbonyl moiety when attached to a carbon atom, a sulfoxide moiety when attached to a sulfur atom and a sulfonyl moiety when two of said terms are attached to a sulfur atom. Whenever a ring or ring system is substituted with an oxo group, the carbon atom to which the oxo is linked is a saturated carbon.

The radical Het is a heterocycle as specified in this specification and claims. Examples of Het comprise, for example, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, pyrrolyl, imidazolyl, oxazolyl, isoxazolyl, thiazinyl, isothiazinyl, thiazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl (including 1,2,3-triazolyl, 1,2,4-triazolyl), tetrazolyl, furanyl, thienyl, pyridyl, pyrimidyl, pyridazinyl, pyrazolyl, triazinyl, and the like. Of interest amongst the Het radicals are those which are non-saturated, in particular those having an aromatic character. Of further interest are those Het radicals having one or two nitrogens.

Each of the Het radicals mentioned in this and the following paragraphs may be optionally substituted with the number and kind of substituents mentioned in the definitions of the compounds of formula (I) or any of the subgroups of compounds of formula (I). Some of the Het radicals mentioned in this and the following paragraphs may be substituted with one, two or three hydroxy substituents. Such hydroxy substituted rings may occur as their tautomeric forms bearing keto groups. For example a 3-hydroxypyridazine moiety can occur in its tautomeric form 2*H*-pyridazin-3-one. . Where Het is piperazinyl, it preferably is substituted in its 4-position by a substituent linked to the 4-nitrogen with a carbon atom, e.g. 4-C₁₋₆alkyl, 4-polyhalo-C₁₋₆alkyl, C₁₋₆alkoxyC₁₋₆alkyl, C₁₋₆alkylcarbonyl, C₃₋₇cycloalkyl.

Interesting Het radicals comprise, for example pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, pyrrolyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl (including 1,2,3-triazolyl, 1,2,4-triazolyl), tetrazolyl, furanyl, thienyl, pyridyl, pyrimidyl, pyridazinyl, pyrazolyl, triazinyl, or any of such heterocycles condensed with a benzene ring, such as indolyl,

indazolyl (in particular 1H-indazolyl), indolyl, quinolyl, tetrahydroquinolyl (in particular 1,2,3,4-tetrahydroquinolyl), isoquinolyl, tetrahydroisoquinolyl (in particular 1,2,3,4-tetrahydroisoquinolyl), quinazolyl, phthalazyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzothiazolyl, benzoxadiazolyl, benzothiadiazolyl, benzofuranyl, benzothienyl.

The Het radicals pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, 4-substituted piperazinyl preferably are linked via their nitrogen atom (i.e. 1-pyrrolidinyl, 1-piperidinyl, 4-thiomorpholinyl, 4-morpholinyl, 1-piperazinyl, 4-substituted 1-piperazinyl).

It should be noted that the radical positions on any molecular moiety used in the definitions may be anywhere on such moiety as long as it is chemically stable.

Radicals used in the definitions of the variables include all possible isomers unless otherwise indicated. For instance pyridyl includes 2-pyridyl, 3-pyridyl and 4-pyridyl; pentyl includes 1-pentyl, 2-pentyl and 3-pentyl.

When any variable occurs more than one time in any constituent, each definition is independent.

Whenever used hereinafter, the term "compounds of formula (I)", or "the present compounds" or similar terms, it is meant to include the compounds of formula (I), their prodrugs, *N*-oxides, addition salts, quaternary amines, metal complexes, and stereochemically isomeric forms. One embodiment comprises the compounds of formula (I) or any subgroup of compounds of formula (I) specified herein, as well as the *N*-oxides, salts, as the possible stereoisomeric forms thereof. Another embodiment comprises the compounds of formula (I) or any subgroup of compounds of formula (I) specified herein, as well as the salts as the possible stereoisomeric forms thereof.

The compounds of formula (I) have several centers of chirality and exist as stereochemically isomeric forms. The term "stereochemically isomeric forms" as used herein defines all the possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of formula (I) may possess.

With reference to the instances where (*R*) or (*S*) is used to designate the absolute configuration of a chiral atom within a substituent, the designation is done taking into consideration the whole compound and not the substituent in isolation.

Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms, which said compound may possess. Said mixture may contain all diastereomers and/or
5 enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of the present invention both in pure form or mixed with each other are intended to be embraced within the scope of the present invention.

Pure stereoisomeric forms of the compounds and intermediates as mentioned herein are
10 defined as isomers substantially free of other enantiomeric or diastereomeric forms of the same basic molecular structure of said compounds or intermediates. In particular, the term "stereoisomerically pure" concerns compounds or intermediates having a stereoisomeric excess of at least 80% (i.e. minimum 90% of one isomer and maximum 10% of the other possible isomers) up to a stereoisomeric excess of 100% (i.e. 100% of
15 one isomer and none of the other), more in particular, compounds or intermediates having a stereoisomeric excess of 90% up to 100%, even more in particular having a stereoisomeric excess of 94% up to 100% and most in particular having a stereoisomeric excess of 97% up to 100%. The terms "enantiomerically pure" and "diastereomerically pure" should be understood in a similar way, but then having
20 regard to the enantiomeric excess, and the diastereomeric excess, respectively, of the mixture in question.

Pure stereoisomeric forms of the compounds and intermediates of this invention may be obtained by the application of art-known procedures. For instance, enantiomers may
25 be separated from each other by the selective crystallization of their diastereomeric salts with optically active acids or bases. Examples thereof are tartaric acid, dibenzoyltartaric acid, ditoluoyltartaric acid and camphosulfonic acid. Alternatively, enantiomers may be separated by chromatographic techniques using chiral stationary phases. Said pure stereochemically isomeric forms may also be derived from the
30 corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably, if a specific stereoisomer is desired, said compound will be synthesized by stereospecific methods of preparation. These methods will advantageously employ enantiomerically pure starting materials.

35 The diastereomeric racemates of the compounds of formula (I) can be obtained separately by conventional methods. Appropriate physical separation methods that

may advantageously be employed are, for example, selective crystallization and chromatography, e.g. column chromatography.

- For some of the compounds of formula (I), their prodrugs, *N*-oxides, salts, solvates, quaternary amines, or metal complexes, and the intermediates used in the preparation thereof, the absolute stereochemical configuration was not experimentally determined. A person skilled in the art is able to determine the absolute configuration of such compounds using art-known methods such as, for example, X-ray diffraction.
- 10 The present invention is also intended to include all isotopes of atoms occurring on the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.
- 15 The term “prodrug” as used throughout this text means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative is the active drug as defined in the compounds of formula (I). The reference by Goodman and Gilman (The Pharmacological Basis of Therapeutics, 8th ed, McGraw-Hill, Int. Ed. 1992, “Biotransformation of Drugs”, p 13–15) describing prodrugs generally is hereby
- 20 incorporated. Prodrugs preferably have excellent aqueous solubility, increased bioavailability and are readily metabolized into the active inhibitors *in vivo*. Prodrugs of a compound of the present invention may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either
- 25 by routine manipulation or *in vivo*, to the parent compound.

- Preferred are pharmaceutically acceptable ester prodrugs that are hydrolysable *in vivo* and are derived from those compounds of formula (I) having a hydroxy or a carboxyl group. An *in vivo* hydrolysable ester is an ester, which is hydrolyzed in the human or animal body to produce the parent acid or alcohol. Suitable pharmaceutically
- 30 acceptable esters for carboxy include C₁₋₆alkoxymethyl esters for example methoxymethyl, C₁₋₆alkanoyloxymethyl esters for example pivaloyloxymethyl, phthalidyl esters, C₃₋₈cycloalkoxycarbonyloxyC₁₋₆alkyl esters for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolen-2-onylmethyl esters for example 5-methyl-1,3-dioxolen-2-onylmethyl; and C₁₋₆alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl which may be formed at any carboxy group in the compounds of this invention.
- 35

An *in vivo* hydrolysable ester of a compound of the formula (I) containing a hydroxy group includes inorganic esters such as phosphate esters and α -acyloxyalkyl ethers and related compounds which as a result of the *in vivo* hydrolysis of the ester breakdown to give the parent hydroxy group. Examples of α -acyloxyalkyl ethers include
5 acetoxymethoxy and 2,2-dimethylpropionyloxy-methoxy. A selection of *in vivo* hydrolysable ester forming groups for hydroxy include alkanoyl, benzoyl, phenylacetyl and substituted benzoyl and phenylacetyl, alkoxycarbonyl (to give alkyl carbonate esters), dialkylcarbamoyl and N-(dialkylaminoethyl)-N-alkylcarbamoyl (to give carbamates), dialkylaminoacetyl and carboxyacetyl. Examples of substituents on
10 benzoyl include morpholino and piperazino linked from a ring nitrogen atom via a methylene group to the 3- or 4-position of the benzoyl ring.

For therapeutic use, salts of the compounds of formula (I) are those wherein the counter-ion is pharmaceutically acceptable. However, salts of acids and bases which
15 are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound. All salts, whether pharmaceutically acceptable or not are included within the ambit of the present invention.

20 The pharmaceutically acceptable acid and base addition salts as mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid and base addition salt forms which the compounds of formula (I) are able to form. The pharmaceutically acceptable acid addition salts can conveniently be obtained by treating the base form with such appropriate acid. Appropriate acids comprise, for example, inorganic acids
25 such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic (i.e. hydroxybutanedioic acid), tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic,
30 salicylic, *p*-aminosalicylic, pamoic and the like acids.

Conversely said salt forms can be converted by treatment with an appropriate base into the free base form.

35 The compounds of formula (I) containing an acidic proton may also be converted into their non-toxic metal or amine addition salt forms by treatment with appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium,

potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, *N*-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

- 5 The term addition salt as used hereinabove also comprises the solvates which the compounds of formula (I) as well as the salts thereof, are able to form. Such solvates are for example hydrates, alcoholates and the like.

10 The term "quaternary amine" as used hereinbefore defines the quaternary ammonium salts which the compounds of formula (I) are able to form by reaction between a basic nitrogen of a compound of formula (I) and an appropriate quaternizing agent, such as, for example, an optionally substituted alkylhalide, arylhalide or arylalkylhalide, e.g. methyl iodide or benzyl iodide. Other reactants with good leaving groups may also be used, such as alkyl trifluoromethanesulfonates, alkyl methanesulfonates, and alkyl
15 *p*-toluenesulfonates. A quaternary amine has a positively charged nitrogen. Pharmaceutically acceptable counterions include chloro, bromo, iodo, trifluoroacetate and acetate. The counterion of choice can be introduced using ion exchange resins.

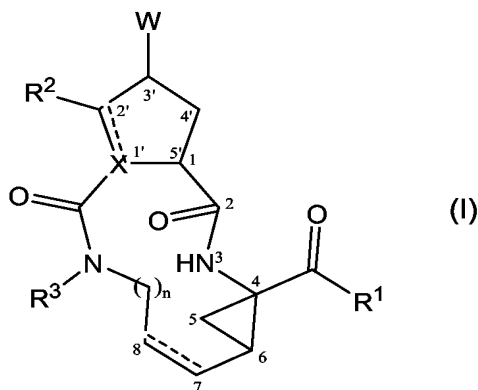
20 The *N*-oxide forms of the present compounds are meant to comprise the compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide.

It will be appreciated that the compounds of formula (I) may have metal binding, chelating, complex forming properties and therefore may exist as metal complexes or metal chelates. Such metalated derivatives of the compounds of formula (I) are
25 intended to be included within the scope of the present invention.

Some of the compounds of formula (I) may also exist in their tautomeric form. Such forms although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

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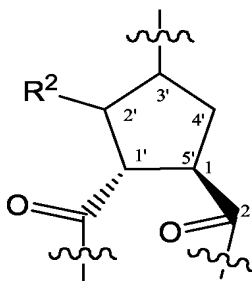
As mentioned above, the compounds of formula (I) have several asymmetric centers. In order to more efficiently refer to each of these asymmetric centers, the numbering system as indicated in the following structural formula will be used.



Asymmetric centers are present at positions 1, 4 and 6 of the macrocycle as well as at the carbon atom 3' in the 5-membered ring, carbon atom 2' when the R² substituent is C₁₋₆alkyl, and at carbon atom 1' when X is CH. Each of these asymmetric centers can occur in their R or S configuration.

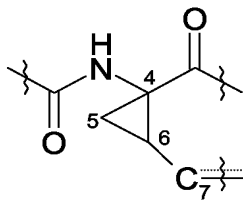
The stereochemistry at position 1 preferably corresponds to that of an L-amino acid configuration, i.e. that of L-proline.

- 10 When X is CH, the 2 carbonyl groups substituted at positions 1' and 5' of the cyclopentane ring preferably are in a trans configuration. The carbonyl substituent at position 5' preferably is in that configuration that corresponds to an L-proline configuration. The carbonyl groups substituted at positions 1' and 5' preferably are as depicted below in the structure of the following formula



15

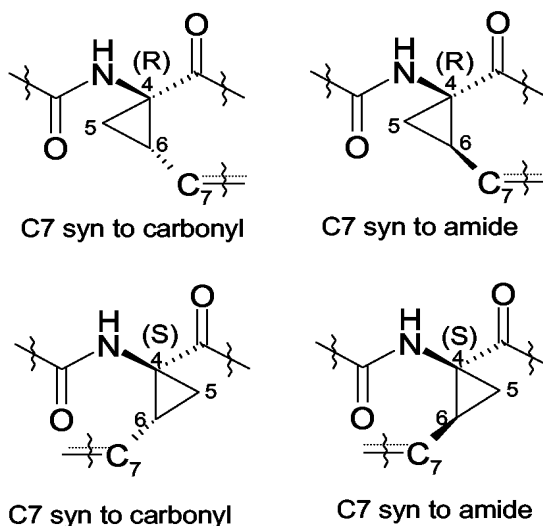
The compounds of formula (I) include a cyclopropyl group as represented in the structural fragment below:



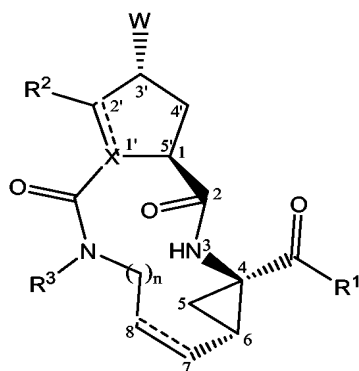
wherein C₇ represents the carbon at position 7 and carbons at position 4 and 6 are asymmetric carbon atoms of the cyclopropane ring.

20

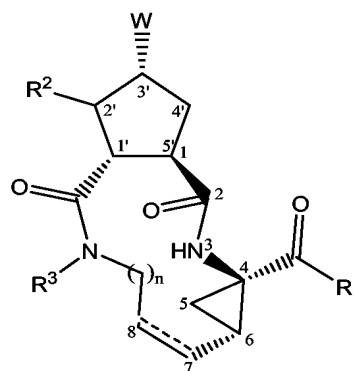
Notwithstanding other possible asymmetric centers at other segments of the compounds of formula (I), the presence of these two asymmetric centers means that the compounds can exist as mixtures of diastereomers, such as the diastereomers of compounds of formula (I) wherein the carbon at position 7 is configured either syn to the carbonyl or syn to the amide as shown below.



- One embodiment concerns compounds of formula (I) wherein the carbon at position 7 is configured syn to the carbonyl. Another embodiment concerns compounds of formula (I) wherein the configuration at the carbon at position 4 is R. A specific subgroup of compounds of formula (I) are those wherein the carbon at position 7 is configured syn to the carbonyl and wherein the configuration at the carbon at position 4 is R.
- The compounds of formula (I) may include a proline residue (when X is N) or a cyclopentyl or cyclopentenyl residue (when X is CH or C). Preferred are the compounds of formula (I) wherein the substituent at the 1 (or 5') position and the W substituent at position 3' are in a trans configuration. Of particular interest are the compounds of formula (I) wherein position 1 has the configuration corresponding to L-proline and the W substituent at position 3' is in a trans configuration in respect of position 1. Preferably the compounds of formula (I) have the stereochemistry as indicated in the structures of formulae (I-a) and (I-b) below:



(I-a)



(I-b)

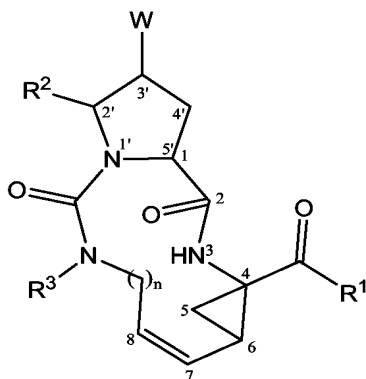
One embodiment of the present invention concerns compounds of formula (I) or of formula (I-a) or of any subgroup of compounds of formula (I), wherein one or more of the following conditions apply:

- (a) R^2 is hydrogen;
- (b) X is nitrogen;
- (c) a double bond is present between carbon atoms 7 and 8.

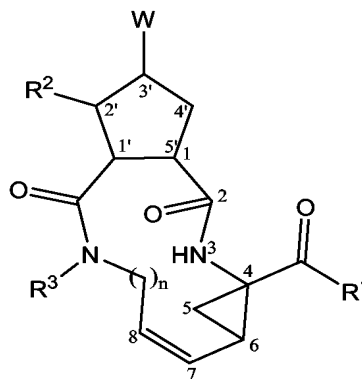
One embodiment of the present invention concerns compounds of formula (I) or of formulae (I-a), (I-b), or of any subgroup of compounds of formula (I), wherein one or more of the following conditions apply:

- (a) R^2 is hydrogen;
- (b) X is CH;
- (c) a double bond is present between carbon atoms 7 and 8.

Particular subgroups of compounds of formula (I) are those represented by the following structural formulae:



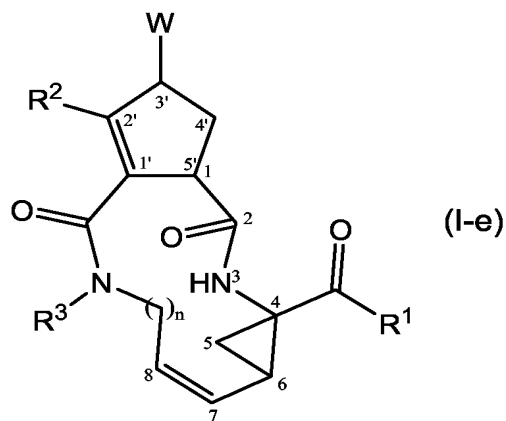
(I-c)



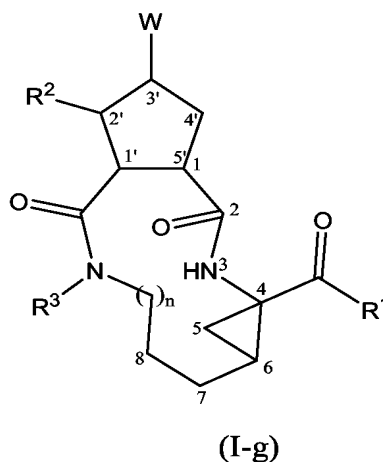
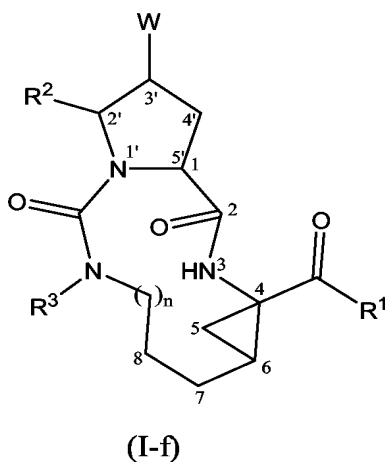
(I-d)

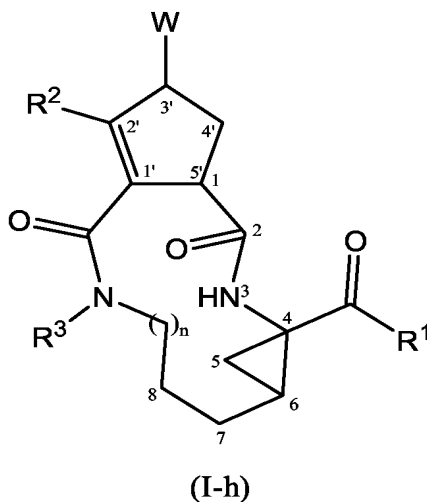
Amongst the compounds of formula (I-c) and (I-d), those having the stereochemical configuration of the compounds of formulae (I-a), and (I-b), respectively, are of particular interest.

- 5 The double bond between carbon atoms 7 and 8 in the compounds of formula (I), or in any subgroup of compounds of formula (I), may be in a *cis* or in a *trans* configuration. Preferably the double bond between carbon atoms 7 and 8 is in a *cis* configuration, as depicted in formulae (I-c) and (I-d).
- 10 A double bond between carbon atoms 1' and 2' may be present in the compounds of formula (I), or in any subgroup of compounds of formula (I), as depicted in formula (I-e) below.



- 15 Yet another particular subgroup of compounds of formula (I) are those represented by the following structural formulae:





Amongst the compounds of formulae (I-f), (I-g) or (I-h), those having the stereochemical configuration of the compounds of formulae (I-a) and (I-b) are of particular interest.

In (I-a), (I-b), (I-c), (I-d), (I-e), (I-f), (I-g) and (I-h), where applicable, W, X, n, R¹, R², and R³ are as specified in the definitions of the compounds of formula (I) or in any of the subgroups of compounds of formula (I) specified herein.

It is to be understood that the above defined subgroups of compounds of formulae (I-a), (I-b), (I-c), (I-d), (I-e), (I-f), (I-g) or (I-h), as well as any other subgroup defined herein, are meant to also comprise any prodrugs, *N*-oxides, addition salts, quaternary amines, metal complexes and stereochemically isomeric forms of such compounds.

When n is 2, the moiety –CH₂– bracketed by “n” corresponds to a ethanediyl in the compounds of formula (I) or in any subgroup of compounds of formula (I). When n is 3, the moiety –CH₂– bracketed by “n” corresponds to a propanediyl in the compounds of formula (I) or in any subgroup of compounds of formula (I). When n is 4, the moiety –CH₂– bracketed by “n” corresponds to a butanediyl in the compounds of formula (I) or in any subgroup of compounds of formula (I). When n is 5, the moiety –CH₂– bracketed by “n” corresponds to a pentanediyl in the compounds of formula (I) or in any subgroup of compounds of formula (I). When n is 6, the moiety –CH₂– bracketed by “n” corresponds to a hexanediyl in the compounds of formula (I) or in any subgroup of compounds of formula (I). Particular subgroups of the compounds of formula (I) are those compounds wherein n is 4 or 5.

Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein

- (a) R^1 is $-OR^{11}$, in particular wherein R^{11} is C_{1-6} alkyl, such as methyl, ethyl, or *tert*-butyl and most preferably where R^{11} is hydrogen; or
- 5 (b) R^1 is $-NHS(=O)_2R^{12}$, in particular wherein R^{12} is C_{1-6} alkyl, C_3-C_7 cycloalkyl, or aryl, e.g. wherein R^{12} is methyl, cyclopropyl, or phenyl; or
- (c) R^1 is $-NHS(=O)_2R^{12}$, in particular wherein R^{12} is C_{3-7} cycloalkyl substituted with C_{1-6} alkyl, preferably wherein R^{12} is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, any of which is substituted with C_{1-4} alkyl, i.e. with methyl, ethyl,
10 propyl, isopropyl, butyl, *tert*-butyl, or isobutyl.

Further embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein R^1 is $-NHS(=O)_2R^{12}$, in particular wherein R^{12} is cyclopropyl substituted with C_{1-4} alkyl, i.e. with methyl, ethyl, propyl, or
15 isopropyl.

Further embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein R^1 is $-NHS(=O)_2R^{12}$, in particular wherein R^{12} is 1-methylcyclopropyl.
20

Further embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein

- (a) R^2 is hydrogen;
- (b) R^2 is C_{1-6} alkyl, preferably methyl.

25

Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein

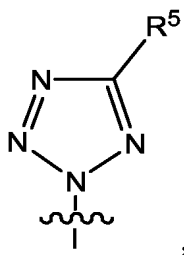
- (a) X is N, C (X being linked via a double bond) or CH (X being linked via a single bond) and R^2 is hydrogen;
- 30 (b) X is C (X being linked via a double bond) and R^2 is C_{1-6} alkyl, preferably methyl.

Further embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein

- (a) R^3 is hydrogen;
- (b) R^3 is C_{1-6} alkyl;
- 35 (d) R^3 is C_{1-6} alkoxy C_{1-6} alkyl or C_{3-7} cycloalkyl.

Preferred embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein R^3 is hydrogen, or C_{1-6} alkyl, more preferably hydrogen or methyl.

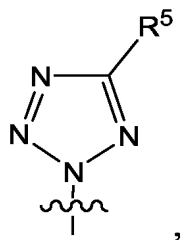
- 5 Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



and R^5 is phenyl optionally substituted with C_{1-6} alkyl or with C_{1-6} alkoxy; thiazolyl optionally substituted with C_{1-6} alkyl; or pyridyl.

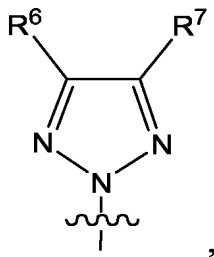
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Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



and R^5 is phenyl, 3-methoxyphenyl, 3,4-dimethoxyphenyl, 4-ethoxyphenyl,
15 4-propoxyphenyl, 4-butoxyphenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl.

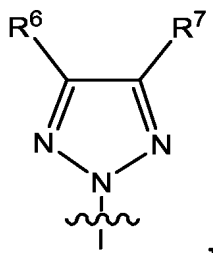
Other subgroups of the compounds of formula (I) are those compounds of formula (I), or any subgroup of compounds of formula (I) specified herein, wherein W is



- 20 wherein R^6 is phenyl optionally substituted with C_{1-6} alkyl or with C_{1-6} alkoxy; thiazolyl optionally substituted with C_{1-6} alkyl; or pyridyl; and

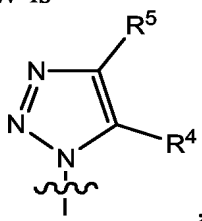
R⁷ is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl.

- Other subgroups of the compounds of formula (I) are those compounds of formula (I), or
 5 any subgroup of compounds of formula (I) specified herein, wherein W is



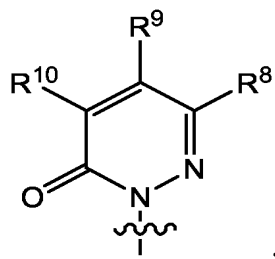
wherein **R⁶** is phenyl, m-methoxyphenyl, 2-pyridyl, 3-pyridyl, or 2-thiazolyl; and **R⁷** is phenyl, p-methoxyphenyl, or 4-ethoxyphenyl.

- 10 Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



- wherein **R⁴** is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl; and
 15 **R⁵** is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl.

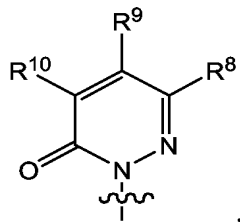
Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



wherein one of R^8 , R^9 , R^{10} is phenyl optionally substituted with C_{1-6} alkyl or with C_{1-6} alkoxy; thiazolyl optionally substituted with C_{1-6} alkyl; or pyridyl; while the other two of R^8 , R^9 , R^{10} independently are hydrogen; phenyl optionally substituted with C_{1-6} alkyl or with C_{1-6} alkoxy; thiazolyl optionally substituted with C_{1-6} alkyl; or pyridyl.

5

Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



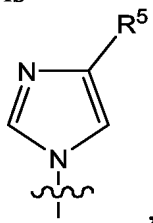
wherein R^8 is hydrogen, 4-methoxyphenyl, or phenyl;

10 wherein R^9 is hydrogen, phenyl, 4-methoxyphenyl, 3-pyridyl, or thiazol-2-yl;

wherein R^{10} is hydrogen, phenyl, 3-pyridyl, or thiazol-2-yl;

wherein each of the three substituents R^8 , R^9 , R^{10} independently is not hydrogen in the same molecule.

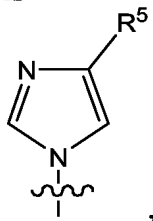
15 Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



wherein R^5 is phenyl optionally substituted with C_{1-6} alkyl or with C_{1-6} alkoxy; thiazolyl optionally substituted with C_{1-6} alkyl; or pyridyl.

20

Embodiments of the invention are compounds of formula (I) or any of the subgroups of compounds of formula (I) wherein W is



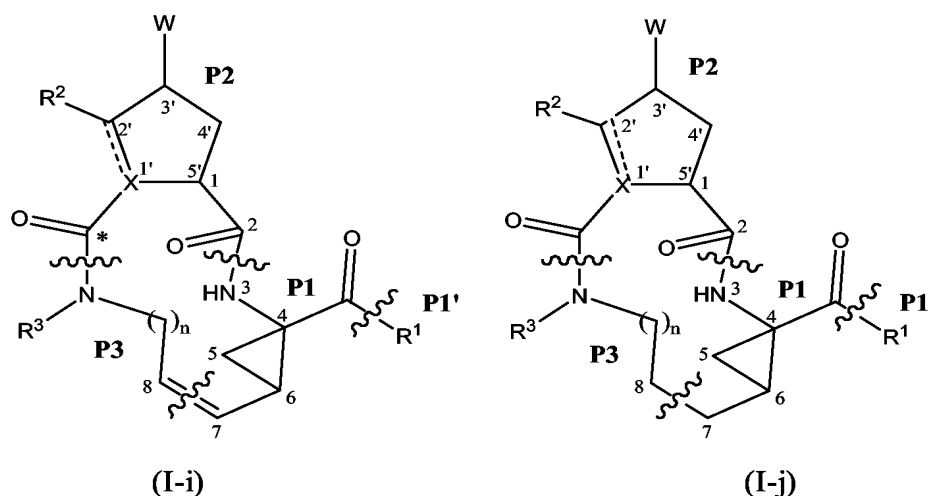
and R^5 is phenyl, 3-methoxyphenyl, 3,4-dimethoxyphenyl, 4-ethoxyphenyl, 4-propoxyphenyl, 4-butoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or thiazol-2-yl.

The compounds of formula (I) consist of three building blocks P1, P2, P3. Building block P1 further contains a P1' tail. The carbonyl group marked with an asterisk in compound (I-c) below may be part of either building block P2 or of building block P3. For reasons of chemistry, building block P2 of the compounds of formula (I) wherein X is C incorporates the carbonyl group attached to the position 1'.

- 10 The linking of building blocks P1 with P2, P2 with P3, and P1 with P1' (when R^1 is $-\text{NH}-\text{SO}_2\text{R}^{12}$) involves forming an amide bond. The linking of blocks P1 and P3 involves double bond formation. The linking of building blocks P1, P2 and P3 to prepare compounds (I-i) or (I-j) can be done in any given sequence. One of the steps involves a cyclization whereby the macrocycle is formed.

15 Represented herebelow are compounds (I-i) which are compounds of formula (I) wherein carbon atoms C7 and C8 are linked by a double bond, and compounds (I-j) which are compounds of formula (I) wherein carbon atoms C7 and C8 are linked by a single bond.

20 The compounds of formula (I-j) can be prepared from the corresponding compounds of formula (I-I) by reducing the double bond in the macrocycle.

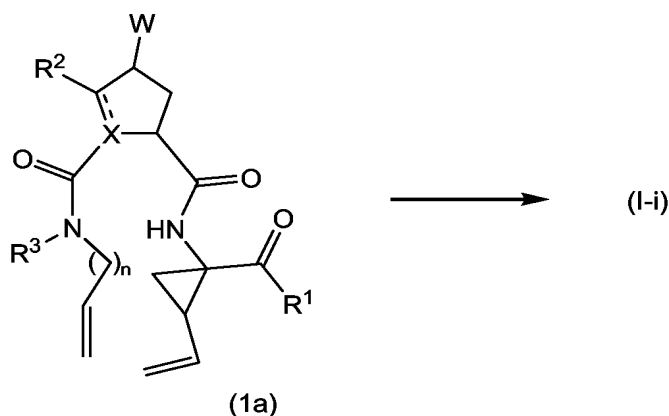


The synthesis procedures described hereinafter are meant to be applicable for as well the racemates, stereochemically pure intermediates or end products, or any stereoisomeric mixtures. The racemates or stereochemical mixtures may be separated into stereoisomeric forms at any stage of the synthesis procedures. In one embodiment,

the intermediates and end products have the stereochemistry specified above in the compounds of formula (I-a) and (I-b).

In one embodiment, compounds (I-i) are prepared by first forming the amide bonds and subsequent forming the double bond linkage between P3 and P1 with concomitant cyclization to the macrocycle.

In a preferred embodiment, compounds (I) wherein the bond between C₇ and C₈ is a double bond, which are compounds of formula (I-i), as defined above, may be prepared by as outlined in the following reaction scheme:



Formation of the macrocycle can be carried out via an olefin metathesis reaction in the presence of a suitable metal catalyst such as e.g. the Ru-based catalyst reported by Miller, S.J., Blackwell, H.E., Grubbs, R.H. J. Am. Chem. Soc. 118, (1996), 9606-9614; Kingsbury, J. S., Harrity, J. P. A., Bonitatebus, P. J., Hoveyda, A. H., J. Am. Chem. Soc. 121, (1999), 791-799; and Huang et al., J. Am. Chem. Soc. 121, (1999), 2674-2678; for example a Hoveyda-Grubbs catalyst.

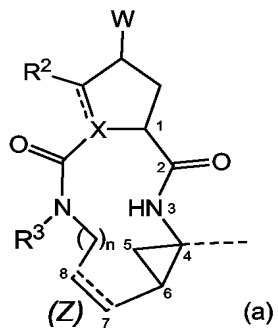
Air-stable ruthenium catalysts such as bis(tricyclohexylphosphine)-3-phenyl-1H-inden-1-ylidene ruthenium chloride (Neolyst M1[®]) or bis(tricyclohexylphosphine)-[(phenylthio)methylene]ruthenium (IV) dichloride can be used. Other catalysts that can be used are Grubbs first and second generation catalysts, i.e. Benzyldiene-bis(tricyclohexylphosphine)dichlororuthenium and (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)-(tricyclohexylphosphine)ruthenium, respectively. Of particular interest are the Hoveyda-Grubbs first and second generation catalysts, which are dichloro(*o*-isopropoxyphenylmethylene)(tricyclohexylphosphine)-ruthenium(II) and 1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-

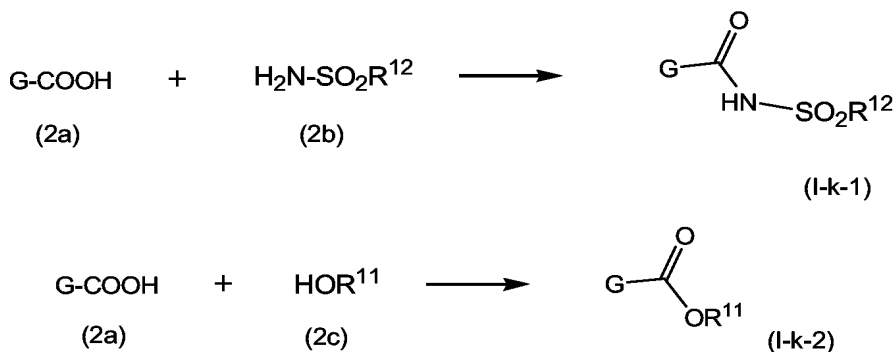
isopropoxyphenylmethylene)ruthenium respectively. Also other catalysts containing other transition metals such as Mo can be used for this reaction.

The metathesis reactions may be conducted in a suitable solvent such as for example ethers, e.g. THF, dioxane; halogenated hydrocarbons, e.g. dichloromethane, CHCl_3 , 1,2-dichloroethane and the like. These reactions are conducted at increased temperatures under nitrogen atmosphere.

Compounds of formula (I) wherein the link between C7 and C8 in the macrocycle is a single bond, i.e. compounds of formula (I-i), can be prepared from the compounds of formula (I-j) by a reduction of the C7-C8 double bond in the compounds of formula (I-i). This reduction may be conducted by catalytic hydrogenation with hydrogen in the presence of a noble metal catalyst such as, for example, Pt, Pd, Rh, Ru or Raney nickel. Of interest is Rh on alumina. The hydrogenation reaction preferably is conducted in a solvent such as, e.g. an alcohol such as methanol, ethanol, or an ether such as THF, or mixtures thereof. Water can also be added to these solvents or solvent mixtures.

The R^1 group can be connected to the P1 building block at any stage of the synthesis, i.e. before or after the cyclization, or before or after the cyclization and reduction as described herein above. The compounds of formula (I) wherein R^1 represents $-\text{NHSO}_2\text{R}^{12}$, said compounds being represented by formula (I-k-1), can be prepared by linking the R^1 group to P1 by forming an amide bond between both moieties. Similarly, the compounds of formula (I) wherein R^1 represents $-\text{OR}^{11}$, i.e. compounds (I-k-2), can be prepared by linking the R^1 group to P1 by forming an ester bond. In one embodiment, the $-\text{OR}^{11}$ groups are introduced in the last step of the synthesis of the compounds (I) as outlined in the following reaction schemes wherein G represents a group:

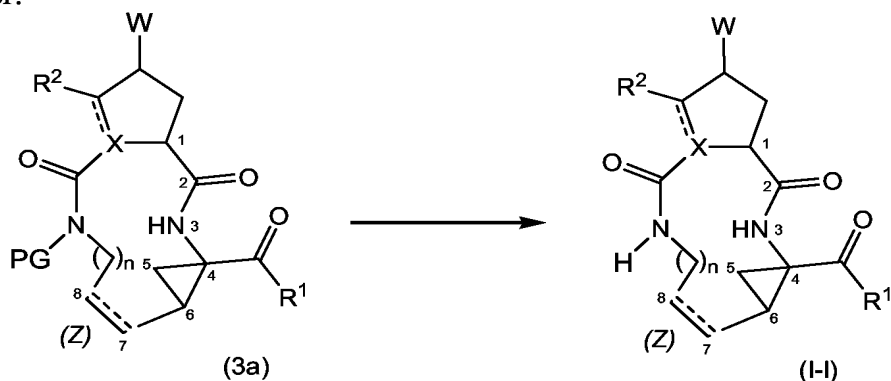




Intermediate (2a) can be coupled with the amine (2b) by an amide forming reaction such as any of the procedures for the formation of an amide bond described hereinafter. In particular, (2a) may be treated with a coupling agent, for example *N,N'*-carbonyl-diimidazole (CDI) or benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (commercially available as PyBOP®), in a solvent like THF, followed by reaction with the desired sulfonamide (2b) in the presence of a base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or diisopropylethylamine. Intermediate (2a) can be coupled with the alcohol (2c) by an ester forming reaction. For example, (2a) and (2c) are reacted together with removal of water either physically, e.g. by azeotropical water removal, or chemically by using a deshydrating agent. Intermediate (2a) can also be converted into an activated form, e.g. an acid chloride (G-CO-Cl) or a mixed acid anhydride (G-CO-O-CO-R, R being e.g. C₁₋₄alkyl or benzyl), and subsequently reacted with the alcohol (2c). The ester forming reactions preferably are conducted in the presence of a base such as an alkali metal carbonate or hydrogen carbonate, e.g. sodium or potassium hydrogen carbonate, or a tertiary amine such as the amines mentioned herein in relation to the amide forming reactions, in particular a trialkylamine, e.g. triethylamine. Solvents that can be used in the ester forming reactions comprise ethers such as THF; halogenated hydrocarbons such as dichloromethane, CH₂Cl₂; hydrocarbons such as toluene; polar aprotic solvents such as DMF, DMSO, DMA; and the like solvents.

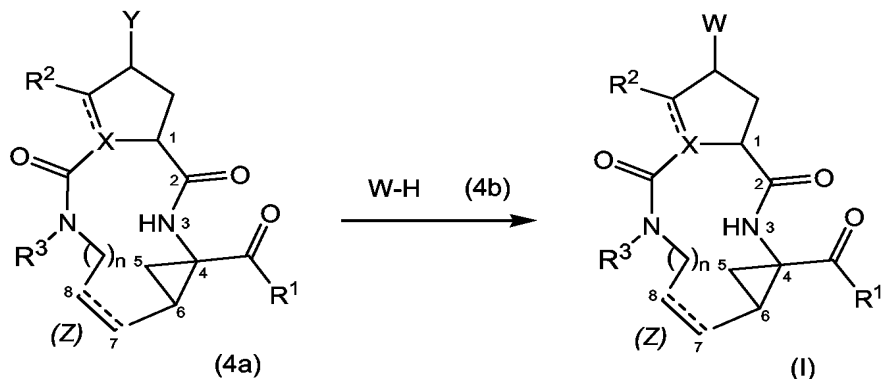
The compounds of formula (I) wherein R³ is hydrogen, said compounds being represented by (I-l), can also be prepared by removal of a protecting group PG, from a corresponding nitrogen-protected intermediate (3a), as in the following reaction scheme. The protecting group PG in particular is any of the nitrogen protecting groups mentioned hereinafter and can be removed using procedures also mentioned

hereinafter:



The starting materials (3a) in the above reaction can be prepared following the procedures for the preparation of compounds of formula (I), but using intermediates wherein the group R^3 is PG.

The compounds of formula (I) can also be prepared by reacting an intermediate (4a) with an heterocycle (4b) as outlined in the following reaction scheme wherein the various radicals have the meanings specified above:



Y in (4a) represents hydroxy or a leaving group such as a halide, e.g. bromide or chloride, or an arylsulfonyl group, e.g. mesylate, triflate or tosylate and the like.

In one embodiment, the reaction of (4a) with (4b) is an O-arylation reaction and Y represents a leaving group. In particular, this reaction is conducted in the presence of a base, preferably a strong base, in a reaction-inert solvent, e.g. one of the solvents mentioned for the formation of an amide bond. In one embodiment, starting material (4a) is reacted with (4b) in the presence of a base which is strong enough to detract a hydrogen from the hydroxy group, for example an alkali of alkaline metal hydride such as LiH or NaH, or an alkali metal alkoxide such as sodium or potassium methoxide or ethoxide, potassium *tert*-butoxide, in a reaction inert solvent like a dipolar aprotic

solvent, e.g. DMA, DMF and the like. The resulting alcoholate is reacted with the arylating agent (4b), wherein Y is a leaving group as mentioned above. The conversion of (4a) to (I) using this type of O-arylation reaction does not change the stereochemical configuration at the carbon bearing the Y or W group.

5

Alternatively, the reaction of (4a) with (4b) can also be conducted via a Mitsunobu reaction (Mitsunobu, 1981, *Synthesis*, January, 1-28; Rano et al., *Tetrahedron Lett.*, 1995, 36, 22, 3779-3792; Krchnak et al., *Tetrahedron Lett.*, 1995, 36, 5, 6193-6196; Richter et al., *Tetrahedron Lett.*, 1994, 35, 27, 4705-4706). This reaction comprises treatment of intermediate (4a) wherein Y is hydroxyl with (4b), in the presence of triphenylphosphine and an activating agent such as a dialkyl azodicarboxylate, e.g. diethyl azodicarboxylate (DEAD), diisopropyl azodicarboxylate (DIAD) or the like. The Mitsunobu reaction changes the stereochemical configuration at the carbon bearing the Y or W group.

15

The starting materials W-H (4b) can be prepared from known or commercially available products. Tetrazoles can be prepared by reacting commercially available nitrile compounds with sodium azide. Triazole derivatives can be prepared by reaction of an alkyne compound and trimethylsilyl azide. Useful alkyne compounds are available either commercially or can be prepared for instance according to the Sonogashira reaction i.e. reaction of a primary alkyne, an aryl halide and triethylamine in the presence of $\text{PdCl}_2(\text{PPh})_3$ and CuI as described for example in A. Elangovan, Y.-H. Wang, T.-I. Ho, *Org. Lett.*, 2003, 5, 1841-1844. The W-substituent can also be modified when attached to the P2 building block, either before or after coupling of the P2 building block to the other building blocks P1 and P3.

25

Further alternatives for the coupling of the W group onto the P2 building block in the preparation of compounds of formula (I) has been extensively described in WO 2004/072243.

30

Alternatively, in order to prepare the compounds of formula (I), first an amide bond between building blocks P2 and P1 is formed, followed by coupling of the P3 building block to the P1 moiety in P1-P2, and a subsequent carbamate or ester bond formation between P3 and the P2 moiety in P2-P1-P3 with concomitant ring closure.

35

Yet another alternative synthetic methodology is the formation of an amide bond between building blocks P2 and P3, followed by the coupling of building block P1 to

the P3 moiety in P3-P2, and a last amide bond formation between P1 and P2 in P1-P3-P2 with concomitant ring closure.

5 Building blocks P1 and P3 can be linked to a P1-P3 sequence. If desired, the double bond linking P1 and P3 may be reduced. The thus formed P1-P3 sequence, either reduced or not, can be coupled to building block P2 and the thus forming sequence P1-P3-P2 subsequently cyclized, by forming an amide bond.

10 Building blocks P1 and P3 in any of the previous approaches can be linked via double bond formation, e.g. by the olefin metathesis reaction described hereinafter, or a Wittig type reaction. If desired, the thus formed double bond can be reduced, similarly as described above for the conversion of (I-i) to (I-j). The double bond can also be reduced at a later stage, i.e. after addition of a third building block, or after formation of the macrocycle. Building blocks P2 and P1 are linked by amide bond formation and P3
15 and P2 are linked by carbamate or ester formation.

The tail P1' can be bonded to the P1 building block at any stage of the synthesis of the compounds of formula (I), for example before or after coupling the building blocks P2 and P1; before or after coupling the P3 building block to P1; or before or after ring
20 closure.

The individual building blocks can first be prepared and subsequently coupled together or alternatively, precursors of the building blocks can be coupled together and modified at a later stage to the desired molecular composition.

25 The functionalities in each of the building blocks may be protected to avoid side reactions.

The formation of amide bonds can be carried out using standard procedures such as
30 those used for coupling amino acids in peptide synthesis. The latter involves the dehydrative coupling of a carboxyl group of one reactant with an amino group of the other reactant to form a linking amide bond. The amide bond formation may be performed by reacting the starting materials in the presence of a coupling agent or by converting the carboxyl functionality into an active form such as an active ester, mixed
35 anhydride or a carboxyl acid chloride or bromide. General descriptions of such coupling reactions and the reagents used therein can be found in general textbooks on peptide chemistry, for example, M. Bodanszky, "Peptide Chemistry", 2nd rev. ed., Springer-Verlag, Berlin, Germany, (1993).

Examples of coupling reactions with amide bond formation include the azide method, mixed carbonic-carboxylic acid anhydride (isobutyl chloroformate) method, the carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide such as *N*-ethyl-*N'*-[(3-dimethylamino)propyl]carbodiimide) method, the active ester method (e.g. *p*-nitrophenyl, *p*-chlorophenyl, trichlorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-hydroxysuccinic imido and the like esters), the Woodward reagent K-method, the 1,1-carbonyldiimidazole (CDI or *N,N'*-carbonyldiimidazole) method, the phosphorus reagents or oxidation-reduction methods. Some of these methods can be enhanced by adding suitable catalysts, e.g. in the carbodiimide method by adding 1-hydroxybenzotriazole, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), or 4-DMAP. Further coupling agents are (benzotriazol-1-yloxy)tris-(dimethylamino) phosphonium hexafluorophosphate, either by itself or in the presence of 1-hydroxybenzotriazole or 4-DMAP; or 2-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, or O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate. These coupling reactions can be performed in either solution (liquid phase) or solid phase.

A preferred amide bond formation is performed employing *N*-ethyloxycarbonyl-2-ethyloxy-1,2-dihydroquinoline (EEDQ) or *N*-isobutyloxy-carbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ). Unlike the classical anhydride procedure, EEDQ and IIDQ do not require base nor low reaction temperatures. Typically, the procedure involves reacting equimolar amounts of the carboxyl and amine components in an organic solvent (a wide variety of solvents can be used). Then EEDQ or IIDQ is added in excess and the mixture is allowed to stir at room temperature.

The coupling reactions preferably are conducted in an inert solvent, such as halogenated hydrocarbons, e.g. dichloromethane, chloroform, dipolar aprotic solvents such as acetonitrile, dimethylformamide, dimethylacetamide, DMSO, HMPT, ethers such as tetrahydrofuran (THF).

In many instances the coupling reactions are done in the presence of a suitable base such as a tertiary amine, e.g. triethylamine, diisopropylethylamine (DIPEA), *N*-methylmorpholine, *N*-methylpyrrolidine, 4-DMAP or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The reaction temperature may range between 0 °C and 50 °C and the reaction time may range between 15 min and 24 h.

The functional groups in the building blocks that are linked together may be protected to avoid formation of undesired bonds. Appropriate protecting groups that can be used are listed for example in Greene, "Protective Groups in Organic Chemistry", John Wiley & Sons, New York (1999) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic Press, New York (1987).

Carboxyl groups can be protected as an ester that can be cleaved off to give the carboxylic acid. Protecting groups that can be used include 1) alkyl esters such as methyl, trimethylsilyl and *tert*-butyl; 2) arylalkyl esters such as benzyl and substituted benzyl; or 3) esters that can be cleaved by a mild base or mild reductive means such as trichloroethyl and phenacyl esters.

Amino groups can be protected by a variety of N-protecting groups, such as:

- 1) acyl groups such as formyl, trifluoroacetyl, phthalyl, and *p*-toluenesulfonyl;
 - 2) aromatic carbamate groups such as benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl (Fmoc);
 - 3) aliphatic carbamate groups such as *tert*-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxy-carbonyl, and allyloxycarbonyl;
 - 4) cyclic alkyl carbamate groups such as cyclopentyloxycarbonyl and adamantyloxycarbonyl;
 - 5) alkyl groups such as triphenylmethyl, benzyl or substituted benzyl such as 4-methoxybenzyl;
 - 6) trialkylsilyl such as trimethylsilyl or *t*.Bu dimethylsilyl; and
 - 7) thiol containing groups such as phenylthiocarbonyl and dithiasuccinoyl.
- Interesting amino protecting groups are Boc and Fmoc.

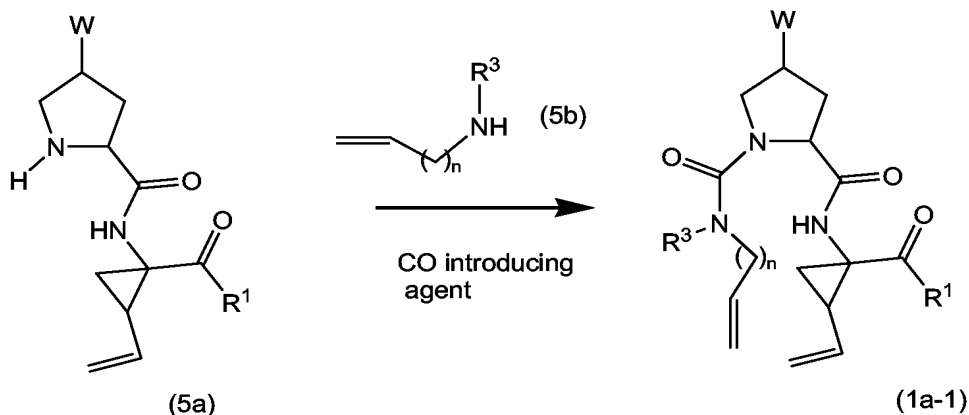
Preferably the amino protecting group is cleaved off prior to the next coupling step. Removal of N-protecting groups can be done following art-known procedures. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane or in ethyl acetate. The resulting ammonium salt is then neutralized either prior to the coupling or in situ with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or acetonitrile or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidine in dimethylformamide, but any secondary amine can be used. The deprotection is carried out at a temperature between 0 °C and room temperature, usually around 15-25 °C, or 20-22 °C.

Other functional groups that can interfere in the coupling reactions of the building blocks may also be protected. For example hydroxyl groups may be protected as benzyl or substituted benzyl ethers, e.g. 4-methoxybenzyl ether, benzoyl or substituted benzoyl esters, e.g. 4-nitrobenzoyl ester, or with trialkylsilyl groups (e.g. trimethylsilyl or *tert*-butyldimethylsilyl).

Further amino groups may be protected by protecting groups that can be cleaved off selectively. For example, when Boc is used as the α -amino protecting group, the following side chain protecting groups are suitable: *p*-toluenesulfonyl (tosyl) moieties can be used to protect further amino groups; benzyl (Bn) ethers can be used to protect hydroxy groups; and benzyl esters can be used to protect further carboxyl groups. Or when Fmoc is chosen for the α -amino protection, usually *tert*-butyl based protecting groups are acceptable. For instance, Boc can be used for further amino groups; *tert*-butyl ethers for hydroxyl groups; and *tert*-butyl esters for further carboxyl groups.

Any of the protecting groups may be removed at any stage of the synthesis procedure but preferably, the protecting groups of any of the functionalities not involved in the reaction steps are removed after completion of the build-up of the macrocycle. Removal of the protecting groups can be done in whatever manner is dictated by the choice of protecting groups, which manners are well known to those skilled in the art.

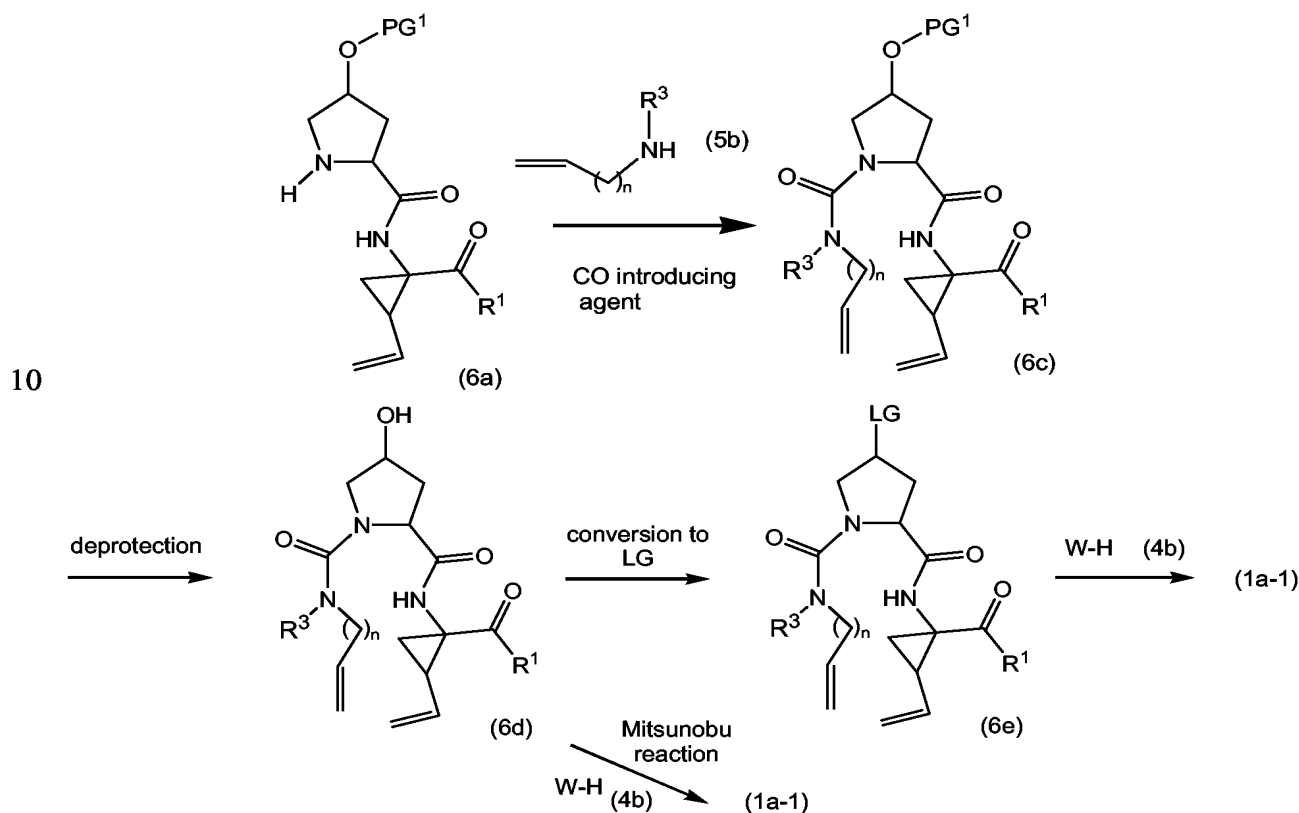
The intermediates of formula (1a) wherein X is N, said intermediates being represented by formula (1a-1), may be prepared starting from intermediates (5a) which are reacted with an alkenamine (5b) in the presence of a carbonyl introducing agent as outlined in the following reaction scheme.



Carbonyl (CO) introducing agents include phosgene, or phosgene derivatives such as carbonyl diimidazole (CDI), and the like. In one embodiment (5a) is reacted with the CO introducing agent in the presence of a suitable base and a solvent, which can be the

bases and solvents used in the amide forming reactions as described above. In a particular embodiment, the base is a hydrogencarbonate, e.g. NaHCO_3 , or a tertiary amine such as triethylamine and the like, and the solvent is an ether or halogenated hydrocarbon, e.g. THF, CH_2Cl_2 , CHCl_3 , and the like. Thereafter, the amine (5b) is added thereby obtaining intermediates (1a-1) as in the above scheme. An alternative route using similar reaction conditions involves first reacting the CO introducing agent with the amine (5b) and then reacting the thus formed intermediate with (5a).

The intermediates (1a-1) can alternatively be prepared as follows:

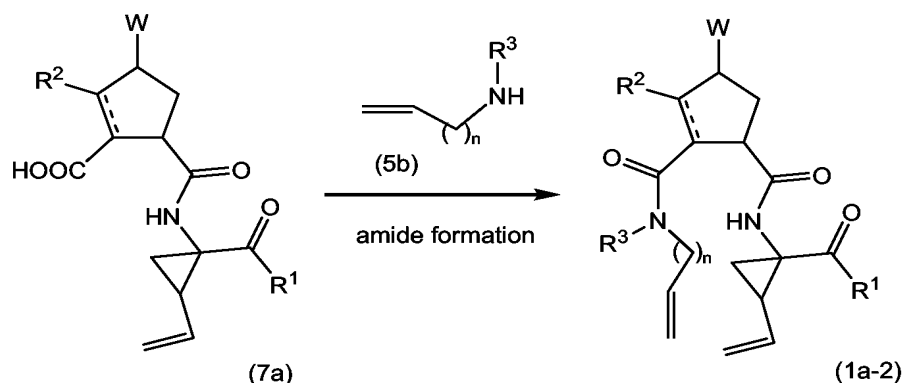


PG¹ is an O-protecting group, which can be any of the groups mentioned herein and in particular is a benzoyl or substituted benzoyl group such as 4-nitrobenzoyl. In the latter instance this group can be removed by reaction with an alkali metal hydroxide (LiOH, NaOH, KOH), in particular where PG¹ is 4-nitrobenzoyl, with LiOH, in an aqueous medium comprising water and a water-soluble organic solvent such as an alkanol (methanol, ethanol) and THF.

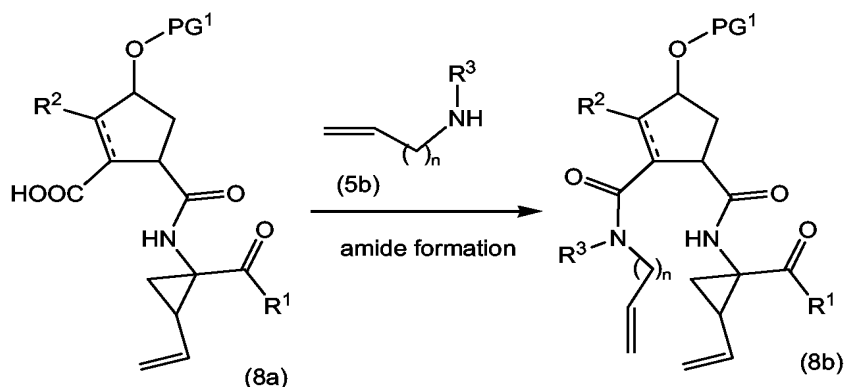
Intermediates (6a) are reacted with (5b) in the presence of a carbonyl introducing agent, similar as described above, and this reaction yields intermediates (6c). These are deprotected, in particular using the reaction conditions mentioned above. The thus

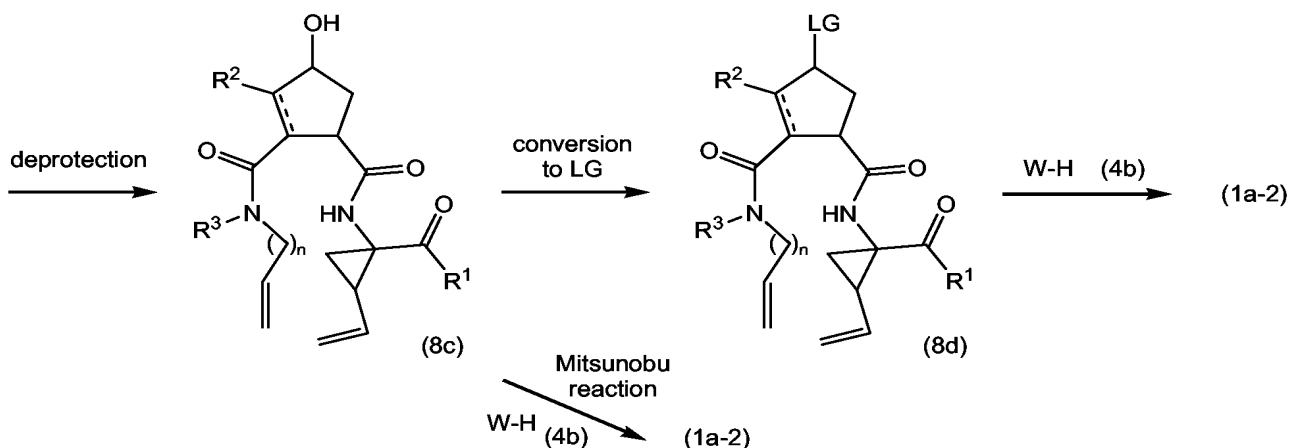
obtained intermediates (6d) can be converted directly to the target intermediates (1a-1) by a Mitsunobu reaction. The resulting alcohol in (6d) can also be converted to a leaving group LG resulting in intermediates (6e), which are reacted with intermediates (4b) as described above for the reaction of (4a) with (4b) and this reaction results in intermediates (1a-1). The alcohol functionality may be converted to a leaving group via art-known procedures, for example by reacting the alcohol with a halogenating reagent such as SOCl_2 or POCl_3 , or by reacting the alcohol with a sulfonyl chloride such as tosyl, mesyl, pp.bromophenyl sulfonyl, trifluoromethylphenyl sulfonyl and the like.

The intermediates of formula (1a) wherein X is C, said intermediates being represented by formula (1a-2), may be prepared by an amide forming reaction starting from intermediates (7a) which are reacted with an amine (5b) as shown in the following reaction scheme, using reaction conditions for preparing amides such as those described above.



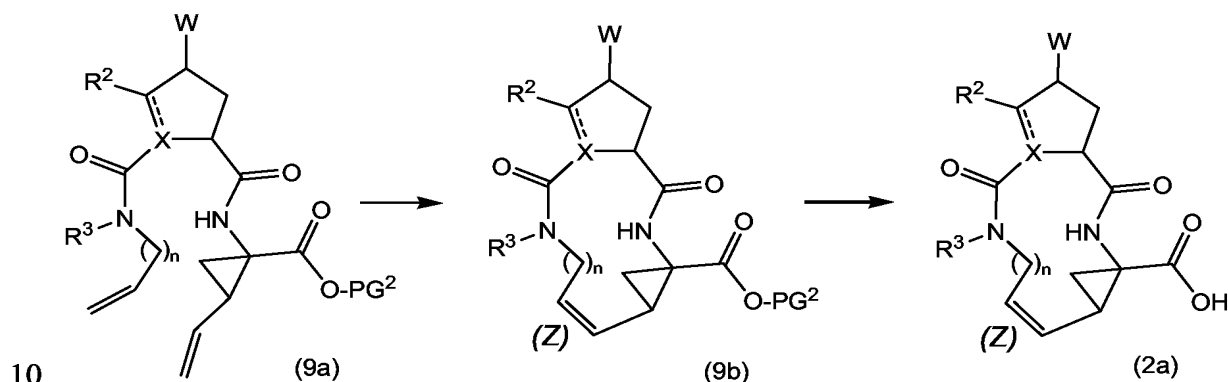
The intermediates (1a-1) can alternatively be prepared as follows:





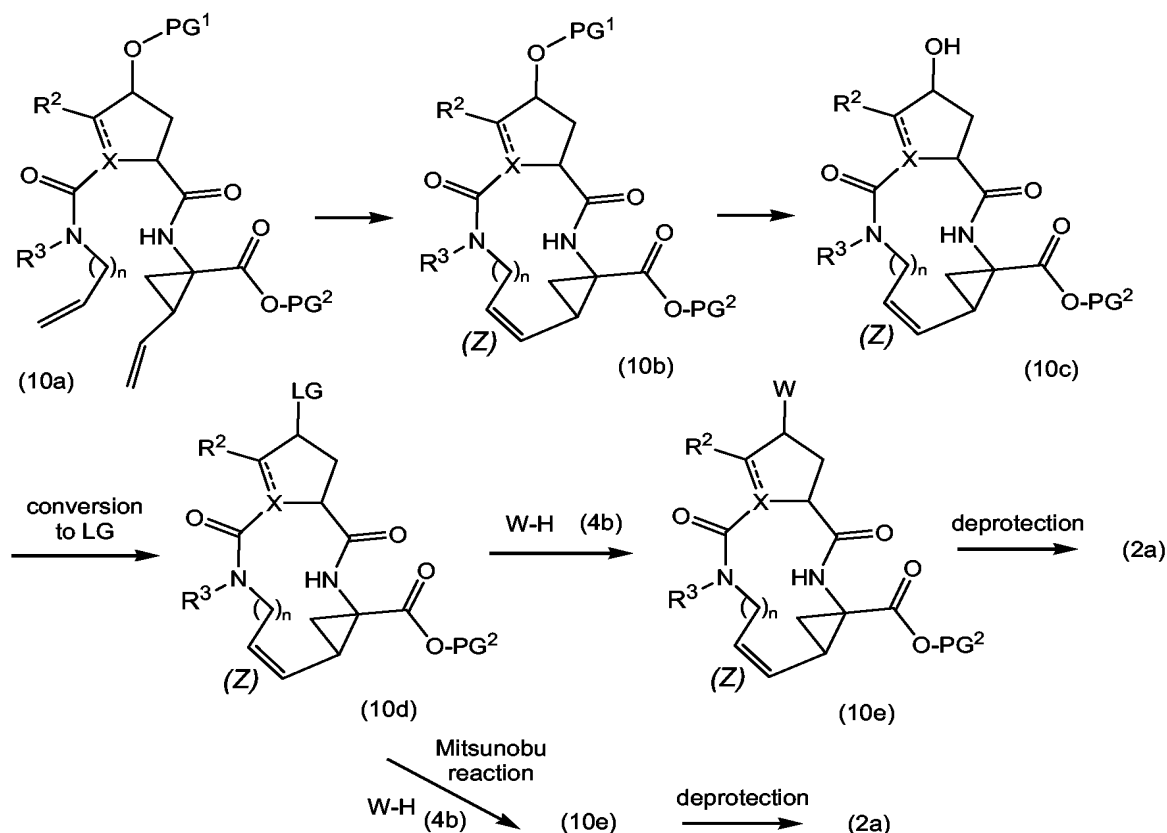
PG¹ is an O-protecting group as described above. The same reaction conditions as described above may be used: amide formation as described above, removal of PG¹ as in the description of the protecting groups, Mitsunobu reaction with (4b) or conversion of the alcohol functionality to a leaving group and introduction of W as in the reactions of (4a) with the reagents (4b).

The intermediates of formula (2a) may be prepared by first cyclizing the open amide (9a) to a macrocyclic ester (9b), which in turn is converted to (2a) as follows:



PG² is a carboxyl protecting group, e.g. one of the carboxyl protecting groups mentioned above, in particular a C₁₋₄alkyl or benzyl ester, e.g. a methyl, ethyl or t.butyl ester. The reaction of (9a) to (9b) is a metathesis reaction and is conducted as described above. The group PG² is removed following procedures also described above. Where PG¹ is a C₁₋₄alkyl ester, it is removed by alkaline hydrolysis, e.g. with NaOH or preferably LiOH, in an aqueous solvent, e.g. a C₁₋₄alkanol/water mixture. A benzyl group can be removed by catalytic hydrogenation.

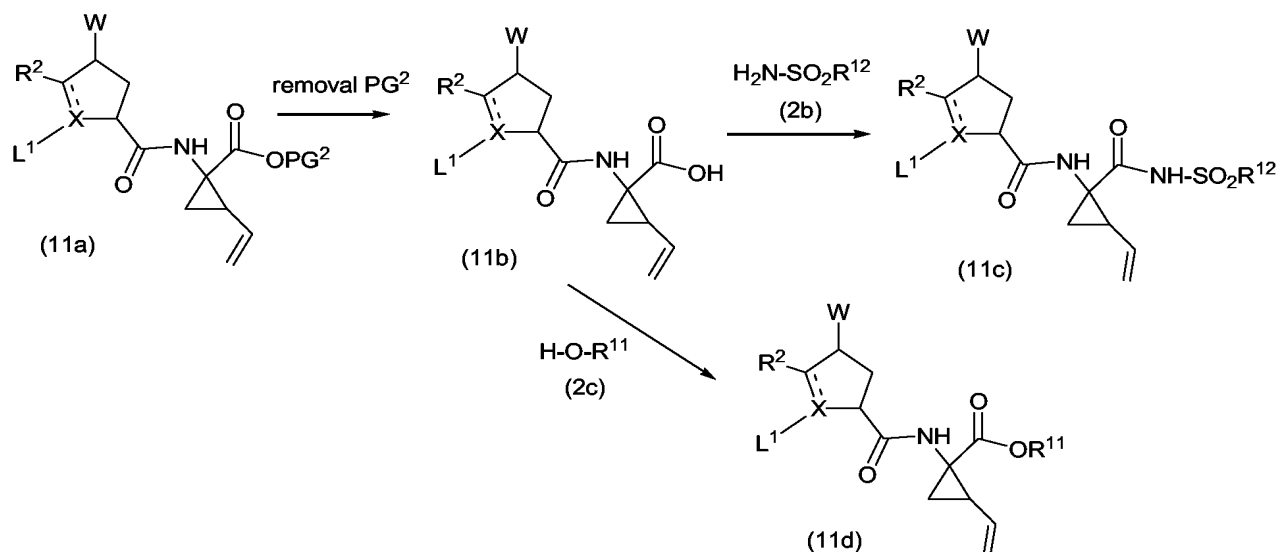
In an alternative synthesis, intermediates (2a) can be prepared as follows:



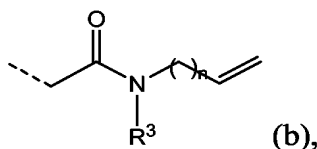
The PG^1 group is selected such that it is selectively cleavable towards PG^2 . PG^2 may be e.g. methyl or ethyl esters, which can be removed by treatment with an alkali metal hydroxide in an aqueous medium, in which case PG^1 e.g. is t.butyl or benzyl. PG^2 may be t.butyl esters removable under weakly acidic conditions or PG^1 may be benzyl esters removable with strong acid or by catalytic hydrogenation, in the latter two cases PG^1 e.g. is a benzoic ester such as a 4-nitrobenzoic ester.

First, intermediates (10a) are cyclized to the macrocyclic esters (10b), the latter are deprotected by removal of the PG^1 group to (10c), which are reacted with intermediates (4b) to intermediates (10e), either directly via a Mitsunobu reaction, or by introducing a LG group and subsequent substitution with W-H. Removal of the carboxyl protecting group PG^2 in (10e) yields (2a). The cyclization, deprotection of PG^1 and PG^2 , conversion to a leaving group LG and the coupling with (4b) are as described above.

Some of the R^1 groups can be introduced at any stage of the synthesis, either as the last step as described above, or earlier, before the macrocycle formation. In the following scheme the group R^1 being $-NH-SO_2R^{12}$ (which is as specified above) is introduced:



In the above scheme, PG^2 is as defined above and L^1 is a P3 group

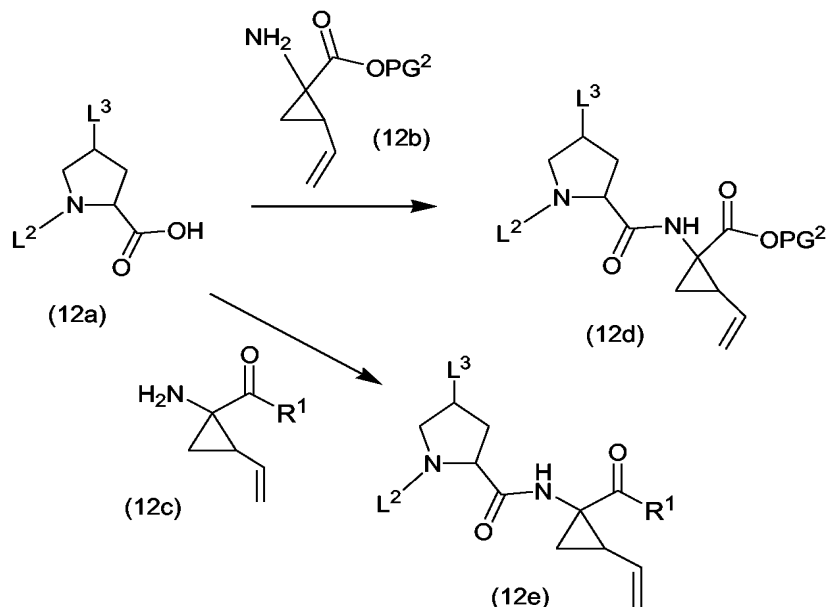


- wherein n and R^3 are as defined above and where X is N , L^1 may also be a nitrogen-protecting group (PG , as defined above) and where X is C , L^1 may also be a group $-\text{COOPG}^{2a}$, wherein the group PG^{2a} is a carboxyl protecting group similar as PG^2 , but wherein PG^{2a} is selectively cleavable towards PG^2 . In one embodiment PG^{2a} is *t*.butyl and PG^2 is methyl or ethyl.

- 10 The intermediates (11c) and (11d) wherein L^1 represents a group (b) correspond to the intermediates (1a) and may be processed further as specified above.

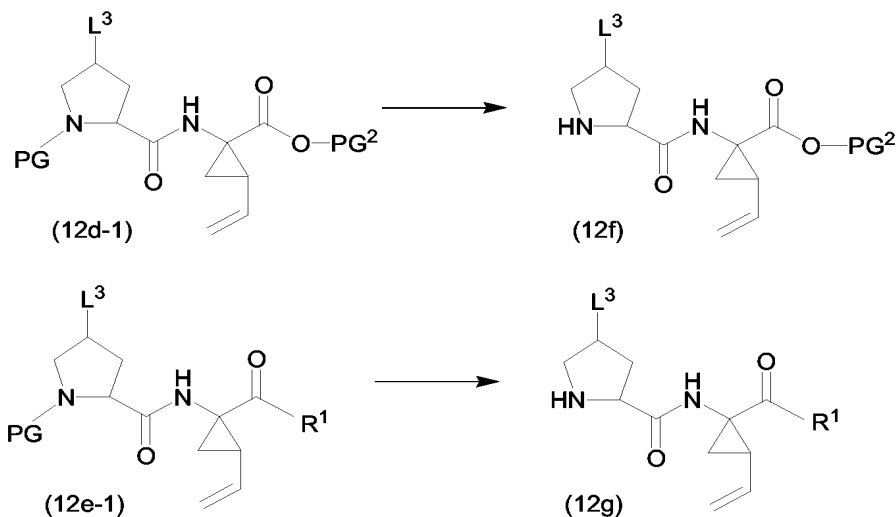
Coupling of P1 and P2 building blocks

- The P1 and P2 building blocks are linked using an amide forming reaction following the procedures described above. The P1 building block may have a carboxyl protecting group PG^2 (as in (12b)) or may already be linked to $\text{P1}'$ group (as in (12c)). L^2 is a N-protecting group (PG), or a group (b), as specified above. L^3 is hydroxy, $-\text{OPG}^1$ or a group $-\text{W}$ as specified above. Where in any of the following reaction schemes L^3 is hydroxy, prior to each reaction step, it may be protected as a group $-\text{OPG}^1$ and, if desired, subsequently deprotected back to a free hydroxy function. Similarly as described above, the hydroxy function may be converted to a group $-\text{W}$.



In the procedure of the above scheme, a cyclopropyl amino acid (12b) or (12c) is coupled to the acid function of the P2 building block (12a) with the formation of an amide linkage, following the procedures described above. Intermediates (12d) or (12e) are obtained. Where in the latter L^2 is a group (b), the resulting products are P3-P2-P1 sequences encompassing some of the intermediates (11c) or (11d) in the previous reaction scheme. Removal of the acid protecting group in (12d), using the appropriate conditions for the protecting group used, followed by coupling with an amine $H_2N-SO_2R^{12}$ (2b) or with HOR^{11} (2c) as described above, again yields the intermediates (12e), wherein $-COR^1$ are amide or ester groups. Where L^2 is a N-protecting group, it can be removed yielding intermediates (5a) or (6a). In one embodiment, PG in this reaction is a BOC group and PG^2 is methyl or ethyl. Where additionally L^3 is hydroxy, the starting material (12a) is Boc-L-hydroxyproline. In a particular embodiment, PG is BOC, PG^2 is methyl or ethyl and L^3 is -W.

In one embodiment, L^2 is a group (b) and these reactions involve coupling P1 to P2-P3, which results in the intermediates (1a-1) or (1a) mentioned above. In another embodiment, L^2 is a N-protecting group PG, which is as specified above, and the coupling reaction results in intermediates (12d-1) or (12e-1), from which the group PG can be removed, using reaction conditions mentioned above, obtaining intermediates (12-f) or respectively (12g), which encompass intermediates (5a) and (6a) as specified above:

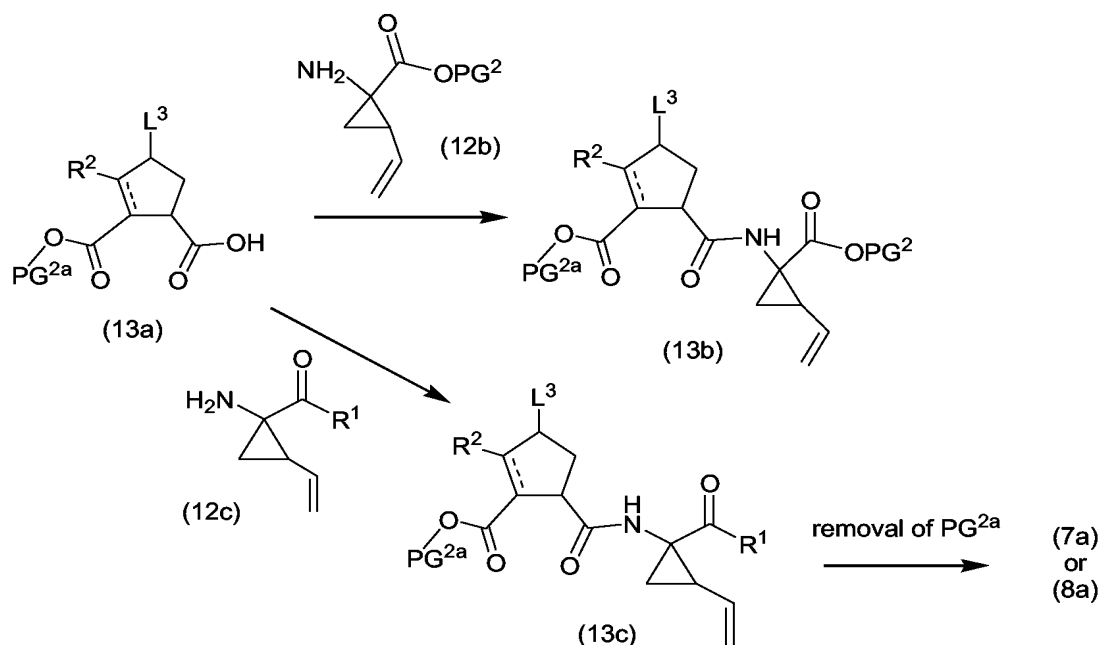


In one embodiment, the group L^3 in the above schemes represents a group $-O-PG^1$ which can be introduced on a starting material (12a) wherein L^3 is hydroxy. In this instance PG^1 is chosen such that it is selectively cleavable towards group L^2 being PG .

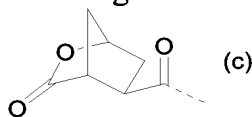
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In a similar way, P2 building blocks wherein X is C, which are cyclopentane or cyclopentene derivatives, can be linked to P1 building blocks as outlined in the following scheme wherein R^1 , R^2 , L^3 , PG^2 and PG^{2a} are carboxyl protecting groups. PG^{2a} typically is chosen such that it is selectively cleavable towards group PG^2 .

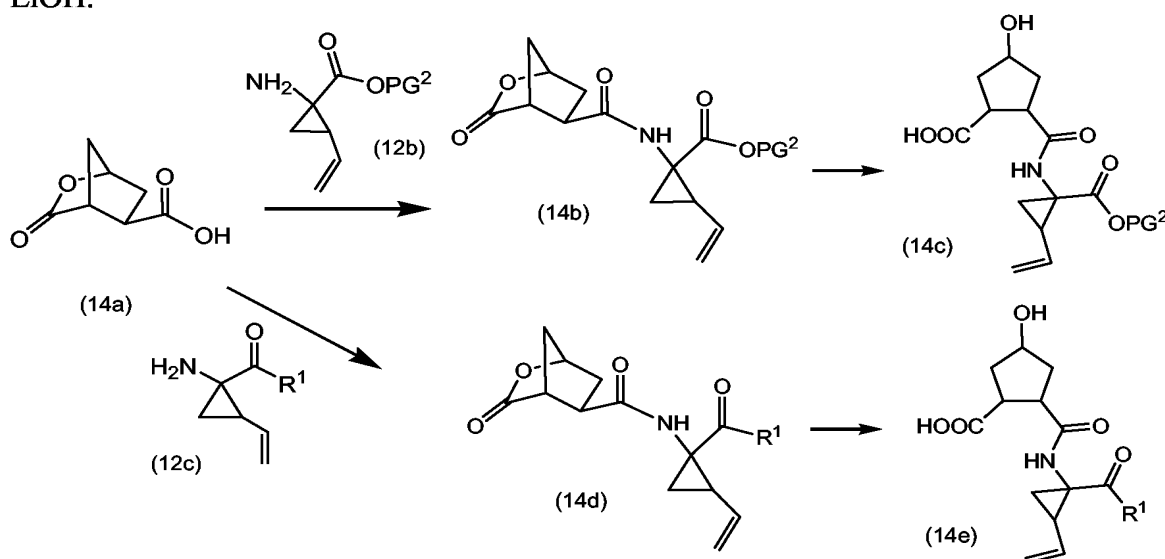
10 Removal of the PG^{2a} group in (13c) yields intermediates (7a) or (8a), which can be reacted with (5b) as described above.



In one particular embodiment, where X is C, R² is H, and where X and the carbon bearing R² are linked by a single bond (P2 being a cyclopentane moiety), PG^{2a} and L³ taken together form a bond and the P2 building block is represented by formula:



- 5 Bicyclic acid (14a) is reacted with (12b) or (12c) similar as described above to (14b) and (14c) respectively, wherein the lactone is opened giving intermediates (14c) and (14e). The lactones can be opened using ester hydrolysis procedures, for example using basic conditions such as an alkali metal hydroxide, e.g. NaOH, KOH, in particular LiOH.

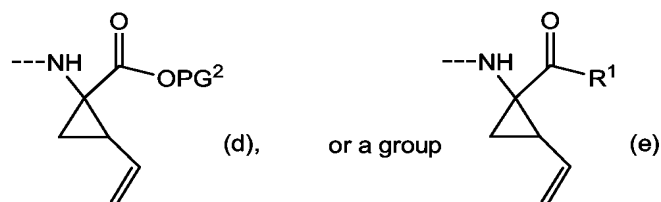


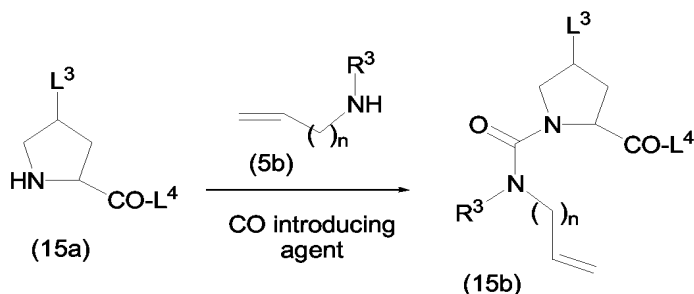
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Intermediates (14c) and (14e) can be processed further as described hereinafter.

Coupling of P3 and P2 building blocks

- For P2 building blocks that have a pyrrolidine moiety, the P3 and P2 or P3 and P2-P1 building blocks are linked using a carbamate forming reaction following the procedures described above for the coupling of (5a) or (6a) with (5b). A general procedure for coupling P2 blocks having a pyrrolidine moiety is represented in the following reaction scheme wherein L³ is as specified above and L⁴ is a group -O-PG², a group

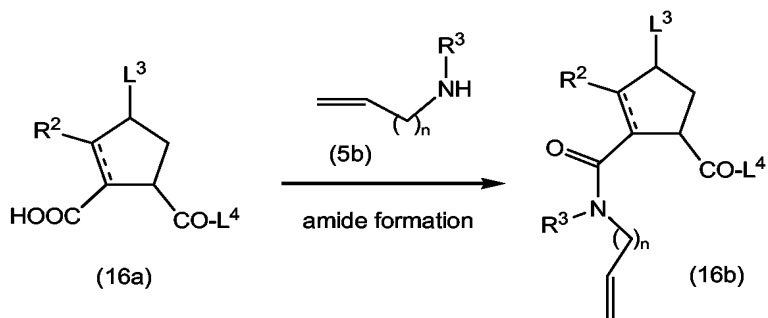




In one embodiment L^4 in (15a) is a group $-OPG^2$, the PG^2 group may be removed and the resulting acid coupled with cyclopropyl amino acids (12a) or (12b), yielding intermediates (12d) or (12e) wherein L^2 is a radical (d) or (e).

5

A general procedure for coupling P3 blocks with a P2 block or a with a P2-P1 block wherein the P2 is a cyclopentane or cyclopentene is shown in the following scheme.



The reactions in the above two schemes are conducted using the same procedures as described above for the reactions of (5a), (7a) or (8a) with (5b) and in particular the above reactions wherein L^4 is a group (d) or (e) correspond to the reactions of (5a), (7a) or (8a) with (5b), described above.

The building blocks P1, P1', P2 and P3 used in the preparation of the compounds of formula (I) can be prepared starting from art-known intermediates. A number of such syntheses are described hereafter in more detail.

The individual building blocks can first be prepared and subsequently coupled together or alternatively, precursors of the building blocks can be coupled together and modified at a later stage to the desired molecular composition.

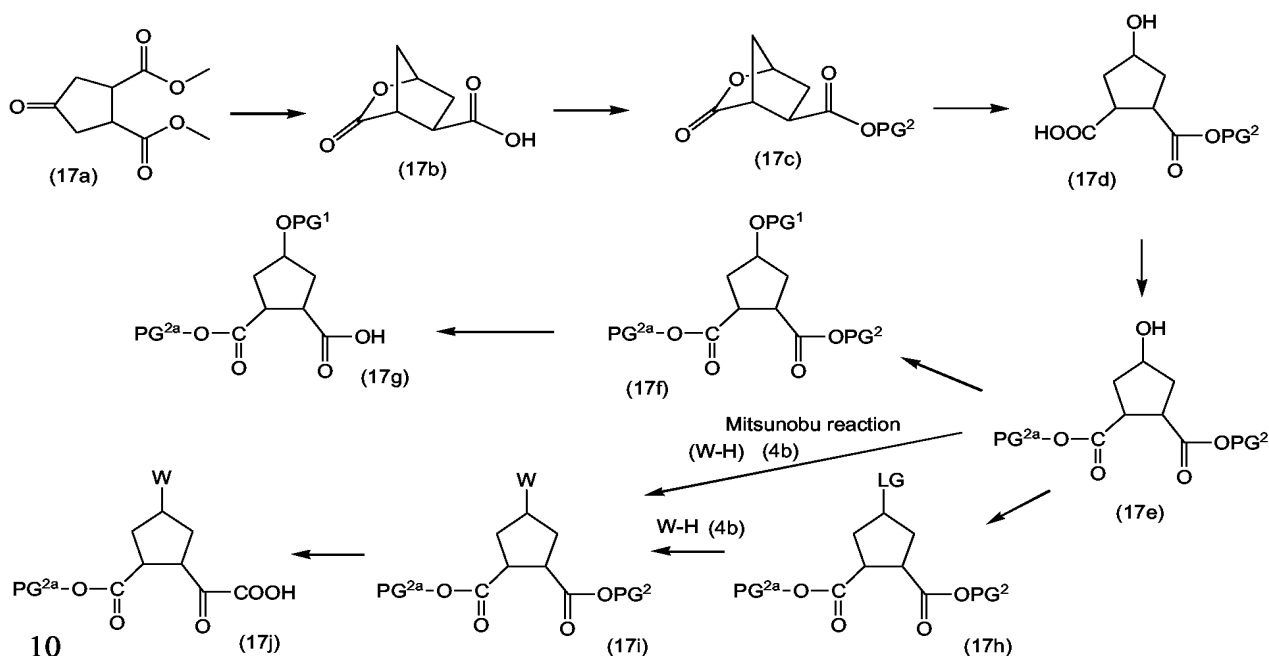
The functionalities in each of the building blocks may be protected to avoid side reactions.

Synthesis of P2 building blocks

The P2 building blocks contain either a pyrrolidine, a cyclopentane, or a cyclopentene moiety substituted with a group –W.

- 5 P2 building blocks containing a pyrrolidine moiety can be derived from commercially available hydroxy proline.

The preparation of P2 building blocks that contain a cyclopentane ring may be performed as shown in the scheme below.

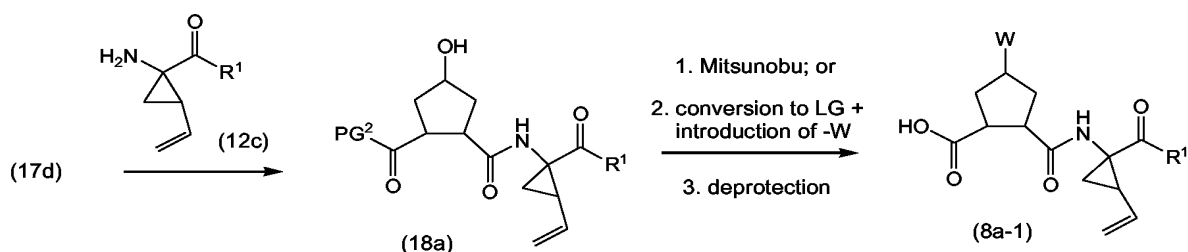


- The bicyclic acid (17b) can be prepared, for example, from 3,4-bis(methoxycarbonyl)-cyclopentanone (17a), as described by Rosenquist et al. in Acta Chem. Scand. 46 (1992) 1127-1129. A first step in this procedure involves the reduction of the keto group with a reducing agent like sodium borohydride in a solvent such as methanol, followed by hydrolysis of the esters and finally ring closure to the bicyclic lactone (17b) using lactone forming procedures, in particular by using acetic anhydride in the presence of a weak base such as pyridine. The carboxylic acid functionality in (17b) can then be protected by introducing an appropriate carboxyl protecting group, such as a group PG², which is as specified above, thus providing bicyclic ester (17c). The group PG² in particular is acid-labile such as a t.butyl group and is introduced e.g. by treatment with isobutene in the presence of a Lewis acid or with di-*tert*-butyl dicarbonate in the presence of a base such as a tertiary amine like dimethylamino-pyridine or triethylamine in a solvent like dichloromethane. Lactone opening of (17c) using reaction conditions described above, in particular with lithium hydroxide, yields

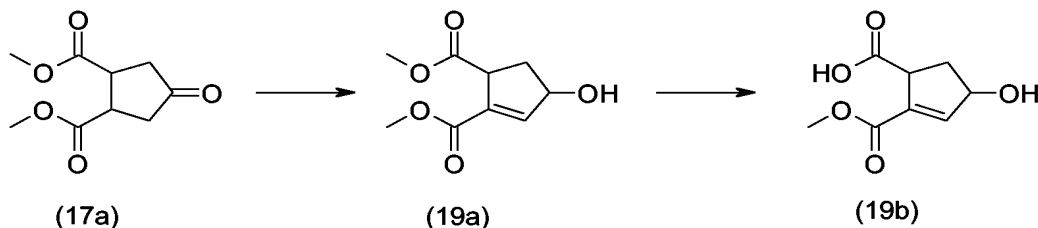
the acid (17d), which can be used further in coupling reactions with P1 building blocks. The free acid in (17d) may also be protected, preferably with an acid protecting group PG^{2a} that is selectively cleavable towards PG^2 , and the hydroxy function may be converted to a group $-OPG^1$ or to a group $-W$. Intermediates (17e) can be converted to intermediates (17i) by introducing a group W using a Mitsunobu reaction. Alternatively, the alcohol functionality in (17e) can be converted to a leaving group and the resulting intermediates (17h) can be reacted with a reagent (4b) to intermediates (17i). The products obtained upon removal of the group PG^2 are intermediates (17g) and (17j) which correspond to intermediates (13a) or (16a) specified above.

Intermediates with specific stereochemistry may be prepared by resolving the intermediates in the above reaction sequence. For example, (17b) may be resolved following art-known procedures, e.g. by salt form action with an optically active base or by chiral chromatography, and the resulting stereoisomers may be processed further as described above. The OH and COOH groups in (17d) are in cis position. Trans analogs can be prepared by inverting the stereochemistry at the carbon bearing the OH function by using specific reagents in the reactions introducing OPG^1 or W that invert the stereochemistry, such as, e.g. by applying a Mitsunobu reaction.

In one embodiment, the intermediates (17d) are coupled to P1 blocks (12b) or (12c), which coupling reactions correspond to the coupling of (13a) or (16a) with the same P1 blocks, using the same conditions. Subsequent introduction of a $-W$ -substituent as described above, followed by removal of the acid protection group PG^2 yields intermediates (8a-1), which are a subclass of the intermediates (7a), or part of the intermediates (16a). The reaction products of the PG^2 removal can be further coupled to a P3 building block. In one embodiment PG^2 in (17d) is t.butyl which can be removed under acidic conditions, e.g. with trifluoroacetic acid.

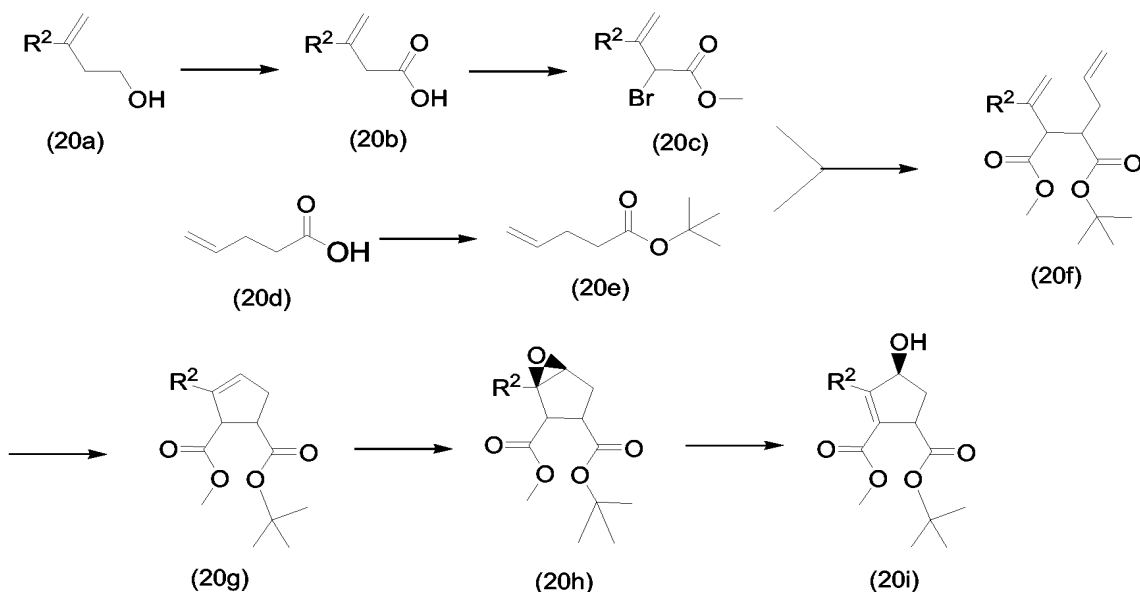


An unsaturated P2 building block, i.e. a cyclopentene ring, may be prepared as illustrated in the scheme below.



A bromination-elimination reaction of 3,4-bis(methoxycarbonyl)cyclopentanone (17a) as described by Dolby et al. in J. Org. Chem. 36 (1971) 1277-1285 followed by reduction of the keto functionality with a reducing agent like sodium borohydride provides the cyclopentenol (19a). Selective ester hydrolysis using for example lithium hydroxide in a solvent like a mixture of dioxane and water, provides the hydroxy substituted monoester cyclopentenol (19b).

An unsaturated P2 building block wherein R^2 can also be other than hydrogen, may be prepared as shown in the scheme below.



Oxidation of commercially available 3-methyl-3-buten-1-ol (20a), in particular by an oxidizing agent like pyridinium chlorochromate, yields (20b), which is converted to the corresponding methyl ester, e.g. by treatment with acetyl chloride in methanol, followed by a bromination reaction with bromine yielding the α -bromo ester (20c). The latter can then be condensed with the alkenyl ester (20e), obtained from (20d) by an ester forming reaction. The ester in (20e) preferably is a t.butyl ester which can be prepared from the corresponding commercially available acid (20d), e.g. by treatment with di-*tert*-butyl dicarbonate in the presence of a base like dimethylaminopyridine. Intermediate (20e) is treated with a base such as lithium diisopropyl amide in a solvent

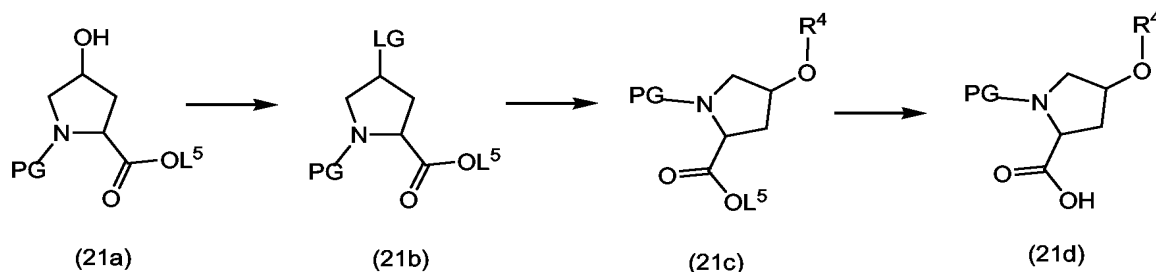
like tetrahydrofuran, and reacted with (20c) to give the alkenyl diester (20f).

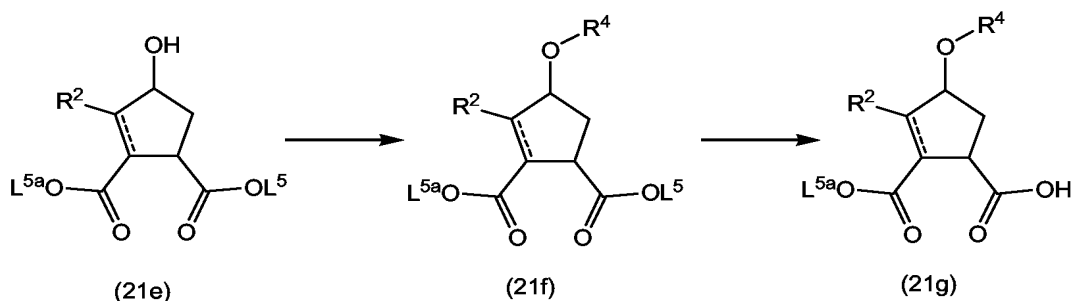
Cyclisation of (20f) by an olefin metathesis reaction, performed as described above, provides cyclopentene derivative (20g). Stereoselective epoxidation of (20g) can be carried out using the Jacobsen asymmetric epoxidation method to obtain epoxide (20h).

5 Finally, an epoxide opening reaction under basic conditions, e.g. by addition of a base, in particular DBN (1,5-diazabicyclo-[4.3.0]non-5-ene), yields the alcohol (20i). Optionally, the double bond in intermediate (20i) can be reduced, for example by catalytic hydrogenation using a catalyst like palladium on carbon, yielding the corresponding cyclopentane compound. The t.butyl ester may be removed to the
10 corresponding acid, which subsequently is coupled to a P1 building block.

The -W group can be introduced on the pyrrolidine, cyclopentane or cyclopentene rings at any convenient stage of the synthesis of the compounds according to the present invention. One approach is to first introduce the -W group to the said rings and subsequently add the other desired building blocks, i.e. P1 (optionally with the P1' tail) and P3, followed by the macrocycle formation. Another approach is to couple the building blocks P2, bearing no -W substituent, with each P1 and P3, and to add the -W group either before or after the macrocycle formation. In the latter procedure, the P2 moieties have a hydroxy group, which may be protected by a hydroxy protecting group PG¹.

W-groups can be introduced on building blocks P2 by converting hydroxy substituted intermediates (21a) to (21b) and reacting the latter with intermediates (4b) as described above for the synthesis of (I) starting from (4a). These reactions are represented in the schemes below, wherein PG is a N-protecting group, and L⁵ and L^{5a} independently from one another, represent hydrogen or a carboxyl protecting group PG² or PG^{2a}. The groups PG, PG² and PG^{2a} are as specified above. Where the groups L⁵ and L^{5a} are PG² or PG^{2a}, they are chosen such that each group is selectively cleavable towards the other. For example, one of L⁵ and L^{5a} may be a methyl or ethyl group and the other a benzyl or t.butyl group.



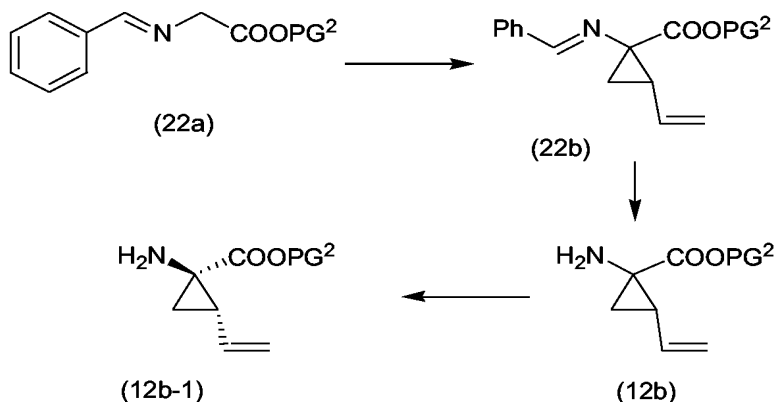


- In one embodiment the group PG is BOC, L^5 is hydrogen and the starting material (21a) is commercially available BOC-hydroxyproline, or any other stereoisomeric form thereof, e.g. BOC-L-hydroxyproline, in particular the trans isomer of the latter. Where L^5 in (21c) is a carboxyl protecting group, it may be removed following procedures described above to (21d). In one embodiment PG in (21c) is Boc and L^5 is a lower alkyl ester, in particular a methyl or ethyl ester. Hydrolysis of the latter ester to the acid can be done by standard procedures, e.g. acid hydrolysis with hydrochloric acid in methanol or with an alkali metal hydroxide such as NaOH, in particular with LiOH. In another embodiment, hydroxy substituted cyclopentane or cyclopentene analogs (21e) are converted to (21f), which, where L^5 and L^{5a} are PG^2 or PG^{2a} , may be converted to the corresponding acids (21g) by removal of the group PG^2 . Removal of PG^{2a} in (21f) leads to similar intermediates.

15 Synthesis of P1 building blocks

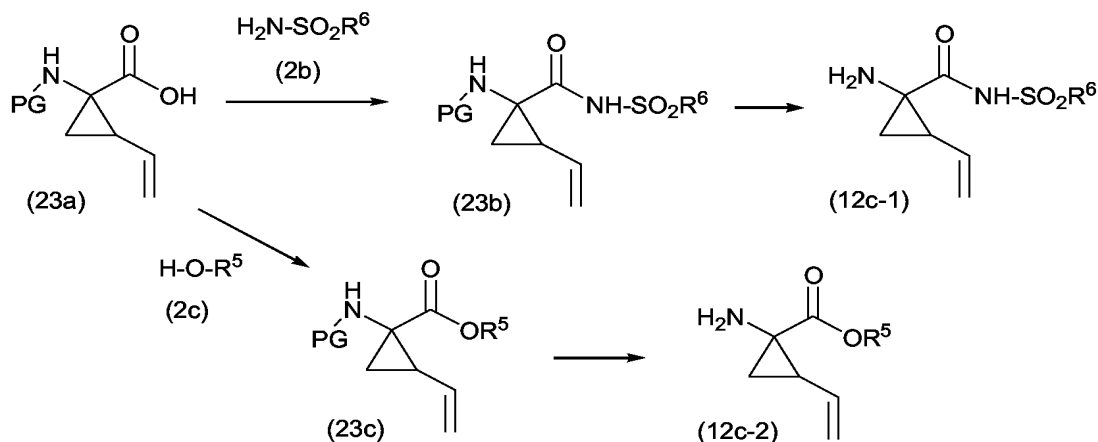
The cyclopropane amino acid used in the preparation of the P1 fragment is commercially available or can be prepared using art-known procedures.

- In particular the amino-vinyl-cyclopropyl ethyl ester (12b) may be obtained according to the procedure described in WO 00/09543 or as illustrated in the following scheme, wherein PG^2 is a carboxyl protecting group as specified above:



Treatment of commercially available or easily obtainable imine (22a) with 1,4-dihalo-butene in presence of a base produces (22b), which after hydrolysis yields cyclopropyl amino acid (12b), having the allyl substituent *syn* to the carboxyl group. Resolution of the enantiomeric mixture (12b) results in (12b-1). The resolution is performed using art-known procedures such as enzymatic separation; crystallization with a chiral acid; or chemical derivatization; or by chiral column chromatography. Intermediates (12b) or (12b-1) may be coupled to the appropriate proline derivatives as described above.

P1 building blocks for the preparation of compounds according to general formula (I) wherein R^1 is $-OR^{11}$ or $-NH-SO_2R^{12}$ can be prepared by reacting amino acids (23a) with the appropriate alcohol or amine respectively under standard conditions for ester or amide formation. Cyclopropyl amino acids (23a) are prepared by introducing a N-protecting group PG, and removal of PG² and the amino acids (23a) are converted to the amides (12c-1) or esters (12c-2), which are subgroups of the intermediates (12c), as outlined in the following reaction scheme, wherein PG is as specified above.



The reaction of (23a) with amine (2b) is an amide forming procedure. The similar reaction with (2c) is an ester forming reaction. Both can be performed following the procedures described above. This reaction yields intermediates (23b) or (23c) from which the amino protecting group is removed by standard methods such as those described above. This in turn results in the desired intermediate (12c-1). Starting materials (23a) may be prepared from the above mentioned intermediates (12b) by first introducing a N-protecting group PG and subsequent removal of the group PG².

In one embodiment the reaction of (23a) with (2b) is done by treatment of the amino acid with a coupling agent, for example N,N'-carbonyl-diimidazole (CDI) or the like, in a solvent like THF followed by reaction with (2b) in the presence of a base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Alternatively the amino acid can be

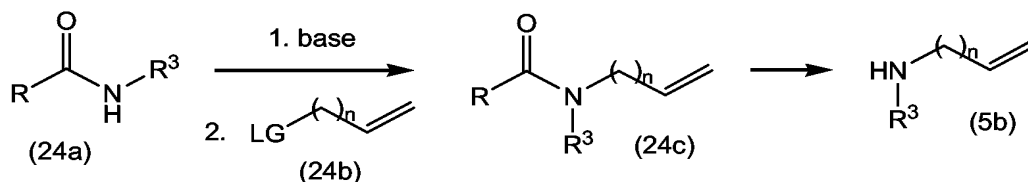
treated with (2b) in the presence of a base like diisopropylethylamine followed by treatment with a coupling agent such as benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (commercially available as PyBOP®) to effect the introduction of the sulfonamide group.

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Intermediates (12c-1) or (12c-2) in turn may be coupled to the appropriate proline, cyclopentane or cyclopentene derivatives as described above.

Synthesis of the P3 building blocks

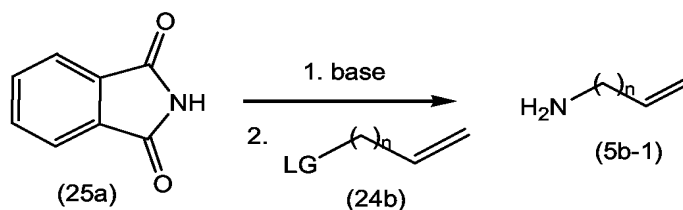
- 10 The P3 building blocks are available commercially or can be prepared according to methodologies known to the skilled in the art. One of these methodologies is shown in the scheme below and uses monoacylated amines, such as trifluoroacetamide or a Boc-protected amine.



- 15 In the above scheme, R together with the CO group forms a N-protecting group, in particular R is *t*-butoxy, trifluoromethyl; R³ and n are as defined above and LG is a leaving group, in particular halogen, e.g. chloro or bromo.

- 20 The monoacylated amines (24a) are treated with a strong base such as sodium hydride and are subsequently reacted with a reagent LG-C₅₋₈alkenyl (24b), in particular haloC₅₋₈alkenyl, to form the corresponding protected amines (24c). Deprotection of (24c) affords (5b), which are building blocks P3. Deprotection will depend on the functional group R, thus if R is *t*-butoxy, deprotection of the corresponding Boc-protected amine can be accomplished with an acidic treatment, e.g. trifluoroacetic acid.
- 25 Alternatively, when R is for instance trifluoromethyl, removal of the R group is accomplished with a base, e.g. sodium hydroxide.

- 30 The following scheme illustrates yet another method for preparing a P3 building block, namely a Gabriel synthesis of primary C₅₋₈alkenylamines, which can be carried out by the treatment of a phthalimide (25a) with a base, such as NaOH or KOH, and with (24b), which is as specified above, followed by hydrolysis of the intermediate N-alkenyl imide to generate a primary C₅₋₈alkenylamine (5b-1).



In the above scheme, n is as defined above.

Compounds of formula (I) may be converted into each other following art-known functional group transformation reactions. For example, amino groups may be N-alkylated, nitro groups reduced to amino groups, a halo atom may be exchanged for another halo.

The compounds of formula (I) may be converted to the corresponding *N*-oxide forms following art-known procedures for converting a trivalent nitrogen into its *N*-oxide form. Said *N*-oxidation reaction may generally be carried out by reacting the starting material of formula (I) with an appropriate organic or inorganic peroxide. Appropriate inorganic peroxides comprise, for example, hydrogen peroxide, alkali metal or earth alkaline metal peroxides, e.g. sodium peroxide, potassium peroxide; appropriate organic peroxides may comprise peroxy acids such as, for example, benzenecarboxoperoxoic acid or halo substituted benzenecarboxoperoxoic acid, e.g. 3-chlorobenzenecarboxoperoxoic acid, peroxyalkanoic acids, e.g. peroxyacetic acid, alkylhydroperoxides, e.g. *tert*-butyl hydroperoxide. Suitable solvents are, for example, water, lower alcohols, e.g. ethanol and the like, hydrocarbons, e.g. toluene, ketones, e.g. 2-butanone, halogenated hydrocarbons, e.g. dichloromethane, and mixtures of such solvents.

Pure stereochemically isomeric forms of the compounds of formula (I) may be obtained by the application of art-known procedures. Diastereomers may be separated by physical methods such as selective crystallization and chromatographic techniques, e.g., counter-current distribution, liquid chromatography and the like.

The compounds of formula (I) may be obtained as racemic mixtures of enantiomers which can be separated from one another following art-known resolution procedures. The racemic compounds of formula (I), which are sufficiently basic or acidic may be converted into the corresponding diastereomeric salt forms by reaction with a suitable chiral acid, respectively chiral base. Said diastereomeric salt forms are subsequently separated, for example, by selective or fractional crystallization and the enantiomers are liberated therefrom by alkali or acid. An alternative manner of separating the enantiomeric forms of the compounds of formula (I) involves liquid chromatography, in

particular liquid chromatography using a chiral stationary phase. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably if a specific stereoisomer is desired, said
5 compound may be synthesized by stereospecific methods of preparation. These methods may advantageously employ enantiomerically pure starting materials.

In a further aspect, the present invention concerns a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I) as
10 specified herein, or a compound of any of the subgroups of compounds of formula (I) as specified herein, and a pharmaceutically acceptable carrier. A therapeutically effective amount in this context is an amount sufficient to prophylactically act against, to stabilize or to reduce viral infection, and in particular HCV viral infection, in infected subjects or subjects being at risk of being infected. In still a further aspect, this
15 invention relates to a process of preparing a pharmaceutical composition as specified herein, which comprises intimately mixing a pharmaceutically acceptable carrier with a therapeutically effective amount of a compound of formula (I), as specified herein, or of a compound of any of the subgroups of compounds of formula (I) as specified herein.

20 Therefore, the compounds of the present invention or any subgroup thereof may be formulated into various pharmaceutical forms for administration purposes. As appropriate compositions there may be cited all compositions usually employed for systemically administering drugs. To prepare the pharmaceutical compositions of this
25 invention, an effective amount of the particular compound, optionally in addition salt form or metal complex, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirable in unitary dosage form suitable, particularly, for
30 administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs, emulsions and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders,
35 disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise

sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case
5 appropriate liquid carriers, suspending agents and the like may be employed. Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in
10 minor proportions, which additives do not introduce a significant deleterious effect on the skin.

The compounds of the present invention may also be administered via oral inhalation or insufflation by means of methods and formulations employed in the art for
15 administration via this way. Thus, in general the compounds of the present invention may be administered to the lungs in the form of a solution, a suspension or a dry powder, a solution being preferred. Any system developed for the delivery of solutions, suspensions or dry powders via oral inhalation or insufflation are suitable for the administration of the present compounds.

20 Thus, the present invention also provides a pharmaceutical composition adapted for administration by inhalation or insufflation through the mouth comprising a compound of formula (I) and a pharmaceutically acceptable carrier. Preferably, the compounds of the present invention are administered via inhalation of a solution in nebulized or
25 aerosolized doses.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suitable as unitary
30 dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such unit dosage forms are tablets (including scored or coated tablets), capsules, pills, suppositories, powder packets, wafers, injectable solutions or suspensions and the like, and segregated multiples thereof.

35 The compounds of formula (I) show antiviral properties. Viral infections and their associated diseases treatable using the compounds and methods of the present invention include those infections brought on by HCV and other pathogenic flaviviruses such as

Yellow fever, Dengue fever (types 1-4), St. Louis encephalitis, Japanese encephalitis, Murray valley encephalitis, West Nile virus and Kunjin virus. The diseases associated with HCV include progressive liver fibrosis, inflammation and necrosis leading to cirrhosis, end-stage liver disease, and HCC; and for the other pathogenic flaviviruses the diseases include yellow fever, dengue fever, hemorrhagic fever and encephalitis. A number of the compounds of this invention moreover are active against mutated strains of HCV. Additionally, many of the compounds of this invention show a favorable pharmacokinetic profile and have attractive properties in terms of bioavailability, including an acceptable half-life, AUC (area under the curve) and peak values and lacking unfavorable phenomena such as insufficient quick onset and tissue retention.

The *in vitro* antiviral activity against HCV of the compounds of formula (I) was tested in a cellular HCV replicon system based on Lohmann et al. (1999) Science 285:110-113, with the further modifications described by Krieger et al. (2001) Journal of Virology 75: 4614-4624, which is further exemplified in the examples section. This model, while not a complete infection model for HCV, is widely accepted as the most robust and efficient model of autonomous HCV RNA replication currently available. Compounds exhibiting anti-HCV activity in this cellular model are considered as candidates for further development in the treatment of HCV infections in mammals. It will be appreciated that it is important to distinguish between compounds that specifically interfere with HCV functions from those that exert cytotoxic or cytostatic effects in the HCV replicon model, and as a consequence cause a decrease in HCV RNA or linked reporter enzyme concentration. Assays are known in the field for the evaluation of cellular cytotoxicity based for example on the activity of mitochondrial enzymes using fluorogenic redox dyes such as resazurin. Furthermore, cellular counter screens exist for the evaluation of non-selective inhibition of linked reporter gene activity, such as firefly luciferase. Appropriate cell types can be equipped by stable transfection with a luciferase reporter gene whose expression is dependent on a constitutively active gene promoter, and such cells can be used as a counter-screen to eliminate non-selective inhibitors.

Due to their antiviral properties, particularly their anti-HCV properties, the compounds of formula (I) or any subgroup thereof, their prodrugs, *N*-oxides, addition salts, quaternary amines, metal complexes and stereochemically isomeric forms, are useful in the treatment of individuals experiencing a viral infection, particularly a HCV infection, and for the prophylaxis of these infections. In general, the compounds of the present invention may be useful in the treatment of warm-blooded animals infected with viruses, in particular flaviviruses such as HCV.

The compounds of the present invention or any subgroup thereof may therefore be used as medicines. Said use as a medicine or method of treatment comprises the systemic administration to viral infected subjects or to subjects susceptible to viral infections of
5 an amount effective to combat the conditions associated with the viral infection, in particular the HCV infection.

The present invention also relates to the use of the present compounds or any subgroup thereof in the manufacture of a medicament for the treatment or the prevention of viral
10 infections, particularly HCV infection.

The present invention furthermore relates to a method of treating a warm-blooded animal infected by a virus, or being at risk of infection by a virus, in particular by HCV, said method comprising the administration of an anti-virally effective amount of
15 a compound of formula (I), as specified herein, or of a compound of any of the subgroups of compounds of formula (I), as specified herein.

Also, the combination of previously known anti-HCV compound, such as, for instance, interferon- α (IFN- α), pegylated interferon- α and/or ribavirin, and a compound of
20 formula (I) can be used as a medicine in a combination therapy. The term “combination therapy” relates to a product containing mandatory (a) a compound of formula (I), and (b) optionally another anti-HCV compound, as a combined preparation for simultaneous, separate or sequential use in treatment of HCV infections, in particular, in the treatment of infections with HCV.

25 Anti-HCV compounds encompass agents selected from an HCV polymerase inhibitor, an HCV protease inhibitor, an inhibitor of another target in the HCV life cycle, and immunomodulatory agent, an antiviral agent, and combinations thereof.

30 HCV polymerase inhibitors include, but are not limited to, NM283 (valopicitabine), R803, JTK-109, JTK-003, HCV-371, HCV-086, HCV-796 and R-1479.

Inhibitors of HCV proteases (NS2-NS3 inhibitors and NS3-NS4A inhibitors) include, but are not limited to, the compounds of WO02/18369 (see, e.g., page 273, lines 9-22
35 and page 274, line 4 to page 276, line 11); BILN-2061, VX-950, GS-9132 (ACH-806), SCH-503034, and SCH-6. Further agents that can be used are those disclosed in WO98/17679, WO00/056331 (Vertex); WO 98/22496 (Roche); WO 99/07734,

(Boehringer Ingelheim), WO 2005/073216, WO 2005073195 (Medivir) and structurally similar agents.

5 Inhibitors of other targets in the HCV life cycle, including NS3 helicase; metallo-
protease inhibitors; antisense oligonucleotide inhibitors, such as ISIS-14803, AVI-4065
and the like; siRNA's such as SIRPLEX-140-N and the like; vector-encoded short
hairpin RNA (shRNA); DNazymes; HCV specific ribozymes such as heptazyme,
RPL13919 and the like; entry inhibitors such as HepeX-C, HuMax-HepC and the like;
10 alpha glucosidase inhibitors such as celgosivir, UT-231B and the like; KPE-02003002;
and BIVN 401.

Immunomodulatory agents include, but are not limited to; natural and recombinant
interferon isoform compounds, including α -interferon, β -interferon, γ -interferon,
 ω -Advaferon®, Infergen®, Humoferon®, Sumiferon MP®, Alfaferone®, IFN-beta®,
15 Feron® and the like; polyethylene glycol derivatized (pegylated) interferon
compounds, such as PEG interferon- α -2a (Pegasys®), PEG interferon- α -2b (PEG-
Intron®), pegylated IFN- α -con1 and the like; long acting formulations and
derivatizations of interferon compounds such as the albumin-fused interferon albuferon
 α and the like; compounds that stimulate the synthesis of interferon in cells, such as
20 resiquimod and the like; interleukins; compounds that enhance the development of type
1 helper T cell response, such as SCV-07 and the like; TOLL-like receptor agonists
such as CpG-10101 (actilon), isatoribine and the like; thymosin α -1; ANA-245; ANA-
246; histamine dihydrochloride; propagermanium; tetrachlorodecaoxide; ampligen;
IMP-321; KRN-7000; antibodies, such as civacir, XTL-6865 and the like; and
25 prophylactic and therapeutic vaccines such as InnoVac C, HCV E1E2/MF59 and the
like.

Other antiviral agents include, but are not limited to, ribavirin, amantadine, viramidine,
nitazoxanide; telbivudine; NOV-205; taribavirin; inhibitors of internal ribosome entry;
30 broad-spectrum viral inhibitors, such as IMPDH inhibitors (e.g., compounds of
US5,807,876, US6,498,178, US6,344,465, US6,054,472, WO97/40028, WO98/40381,
WO00/56331, and mycophenolic acid and derivatives thereof, and including, but not
limited to VX-950, merimepodib (VX-497), VX-148, and/or VX-944); or combinations
of any of the above.

35

Thus, to combat or treat HCV infections, the compounds of formula (I) may be
co-administered in combination with for instance, interferon- α (IFN- α), pegylated
interferon- α and/or ribavirin, as well as therapeutics based on antibodies targeted

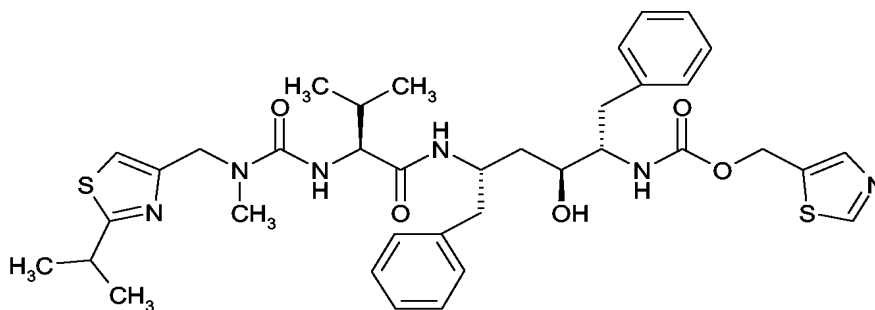
against HCV epitopes, small interfering RNA (Si RNA), ribozymes, DNazymes, antisense RNA, small molecule antagonists of for instance NS3 protease, NS3 helicase and NS5B polymerase.

- 5 Accordingly, the present invention relates to the use of a compound of formula (I) or any subgroup thereof as defined above for the manufacture of a medicament useful for inhibiting HCV activity in a mammal infected with HCV viruses, wherein said medicament is used in a combination therapy, said combination therapy preferably comprising a compound of formula (I) and another HCV inhibitory compound, e.g.
10 (pegylated) IFN- α and/or ribavirin.

In still another aspect there are provided combinations of a compound of formula (I) as specified herein and an anti-HIV compound. The latter preferably are those HIV inhibitors that have a positive effect on drug metabolism and/or pharmacokinetics that
15 improve bioavailability. An example of such an HIV inhibitor is ritonavir.

As such, the present invention further provides a combination comprising (a) an HCV NS3/4a protease inhibitor of formula (I) or a pharmaceutically acceptable salt thereof; and (b) ritonavir or a pharmaceutically acceptable salt thereof.
20

The compound ritonavir, and pharmaceutically acceptable salts thereof, and methods for its preparation are described in WO94/14436. For preferred dosage forms of ritonavir, see US6,037, 157, and the documents cited therein: US5,484, 801, US08/402,690, and WO95/07696 and WO95/09614. Ritonavir has the following
25 formula:



In a further embodiment, the combination comprising (a) an HCV NS3/4a protease inhibitor of formula (I) or a pharmaceutically acceptable salt thereof; and (b) ritonavir or a pharmaceutically acceptable salt thereof; further comprises an additional anti-HCV
30 compound selected from the compounds as described herein.

In one embodiment of the present invention there is provided a process for preparing a combination as described herein, comprising the step of combining an HCV NS3/4a protease inhibitor of formula (I) or a pharmaceutically acceptable salt thereof, and ritonavir or a pharmaceutically acceptable salt thereof. An alternative embodiment of this invention provides a process wherein the combination comprises one or more additional agent as described herein.

The combinations of the present invention may be used as medicaments. Said use as a medicine or method of treatment comprises the systemic administration to HCV-infected subjects of an amount effective to combat the conditions associated with HCV and other pathogenic flavi- and pestiviruses. Consequently, the combinations of the present invention can be used in the manufacture of a medicament useful for treating, preventing or combating infection or disease associated with HCV infection in a mammal, in particular for treating conditions associated with HCV and other pathogenic flavi- and pestiviruses.

In one embodiment of the present invention there is provided a pharmaceutical composition comprising a combination according to any one of the embodiments described herein and a pharmaceutically acceptable excipient. In particular, the present invention provides a pharmaceutical composition comprising (a) a therapeutically effective amount of an HCV NS3/4a protease inhibitor of the formula (I) or a pharmaceutically acceptable salt thereof, (b) a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof, and (c) a pharmaceutically acceptable excipient. Optionally, the pharmaceutical composition further comprises an additional agent selected from an HCV polymerase inhibitor, an HCV protease inhibitor, an inhibitor of another target in the HCV life cycle, and immunomodulatory agent, an antiviral agent, and combinations thereof.

The compositions may be formulated into suitable pharmaceutical dosage forms such as the dosage forms described above. Each of the active ingredients may be formulated separately and the formulations may be co-administered or one formulation containing both and if desired further active ingredients may be provided.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients, as well as any product which results, directly or indirectly, from the combination of the specified ingredients.

In one embodiment the combinations provided herein may also be formulated as a combined preparation for simultaneous, separate or sequential use in HIV therapy. In

such a case, the compound of general formula (I) or any subgroup thereof, is formulated in a pharmaceutical composition containing other pharmaceutically acceptable excipients, and ritonavir is formulated separately in a pharmaceutical composition containing other pharmaceutically acceptable excipients. Conveniently, these two separate pharmaceutical compositions can be part of a kit for simultaneous, separate or sequential use.

Thus, the individual components of the combination of the present invention can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The present invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly. In a preferred embodiment, the separate dosage forms are administered about simultaneously.

In one embodiment, the combination of the present invention contains an amount of ritonavir, or a pharmaceutically acceptable salt thereof, which is sufficient to clinically improve the bioavailability of the HCV NS3/4a protease inhibitor of formula (I) relative to the bioavailability when said HCV NS3/4a protease inhibitor of formula (I) is administered alone.

In another embodiment, the combination of the present invention contains an amount of ritonavir, or a pharmaceutically acceptable salt thereof, which is sufficient to increase at least one of the pharmacokinetic variables of the HCV NS3/4a protease inhibitor of formula (I) selected from $t_{1/2}$, C_{min} , C_{max} , C_{ss} , AUC at 12 hours, or AUC at 24 hours, relative to said at least one pharmacokinetic variable when the HCV NS3/4a protease inhibitor of formula (I) is administered alone.

A further embodiment relates to a method for improving the bioavailability of a HCV NS3/4a protease inhibitor comprising administering to an individual in need of such improvement a combination as defined herein, comprising a therapeutically effective amount of each component of said combination.

In a further embodiment, the invention relates to the use of ritonavir or a pharmaceutically acceptable salt thereof, as an improver of at least one of the pharmacokinetic variables of a HCV NS3/4a protease inhibitor of formula (I) selected from $t_{1/2}$, C_{min} , C_{max} , C_{ss} , AUC at 12 hours, or AUC at 24 hours; with the proviso that said use is not practised in the human or animal body.

The term “individual” as used herein refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

Bioavailability is defined as the fraction of administered dose reaching systemic
5 circulation. $t_{1/2}$ represents the half life or time taken for the plasma concentration to fall
to half its original value. C_{ss} is the steady state concentration, i.e. the concentration at
which the rate of input of drug equals the rate of elimination. C_{min} is defined as the
lowest (minimum) concentration measured during the dosing interval. C_{max} , represents
the highest (maximum) concentration measured during the dosing interval. AUC is
10 defined as the area under the plasma concentration-time curve for a defined period of
time.

The combinations of this invention can be administered to humans in dosage ranges
specific for each component comprised in said combinations. The components
15 comprised in said combinations can be administered together or separately. The
NS3/4a protease inhibitors of formula (I) or any subgroup thereof, and ritonavir or a
pharmaceutically acceptable salt or ester thereof, may have dosage levels of the order
of 0.02 to 5.0 grams-per-day.

20 When the HCV NS3/4a protease inhibitor of formula (I) and ritonavir are administered
in combination, the weight ratio of the HCV NS3/4a protease inhibitor of formula (I) to
ritonavir is suitably in the range of from about 40:1 to about 1:15, or from about 30:1 to
about 1:15, or from about 15:1 to about 1:15, typically from about 10:1 to about 1:10,
and more typically from about 8:1 to about 1:8. Also useful are weight ratios of the
25 HCV NS3/4a protease inhibitors of formula (I) to ritonavir ranging from about 6:1 to
about 1:6, or from about 4:1 to about 1:4, or from about 3:1 to about 1:3, or from about
2:1 to about 1:2, or from about 1.5:1 to about 1:1.5. In one aspect, the amount by
weight of the HCV NS3/4a protease inhibitors of formula (I) is equal to or greater than
that of ritonavir, wherein the weight ratio of the HCV NS3/4a protease inhibitor of
30 formula (I) to ritonavir is suitably in the range of from about 1:1 to about 15:1,
typically from about 1:1 to about 10:1, and more typically from about 1:1 to about 8:
1. Also useful are weight ratios of the HCV NS3/4a protease inhibitor of formula (I) to
ritonavir ranging from about 1:1 to about 6:1, or from about 1:1 to about 5:1, or from
about 1:1 to about 4:1, or from about 3:2 to about 3:1, or from about 1:1 to about 2:1
35 or from about 1:1 to about 1.5:1.

The term “therapeutically effective amount” as used herein means that amount of active
compound or component or pharmaceutical agent that elicits the biological or

medicinal response in a tissue, system, animal or human that is being sought, in the light of the present invention, by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease being treated.

Since the instant invention refers to combinations comprising two or more agents, the
5 “therapeutically effective amount” is that amount of the agents taken together so that the combined effect elicits the desired biological or medicinal response. For example, the therapeutically effective amount of a composition comprising (a) the compound of formula (I) and (b) ritonavir, would be the amount of the compound of formula (I) and the amount of ritonavir that when taken together have a combined effect that is
10 therapeutically effective.

In general it is contemplated that an antiviral effective daily amount would be from 0.01 mg/kg to 500 mg/kg body weight, more preferably from 0.1 mg/kg to 50 mg/kg body weight. It may be appropriate to administer the required dose as one, two, three,
15 four or more (sub-)doses at appropriate intervals throughout the day. Said (sub-)doses may be formulated as unit dosage forms, for example, containing 1 to 1000 mg, and in particular 5 to 200 mg of active ingredient per unit dosage form.

The exact dosage and frequency of administration depends on the particular compound
20 of formula (I) used, the particular condition being treated, the severity of the condition being treated, the age, weight, sex, extent of disorder and general physical condition of the particular patient as well as other medication the individual may be taking, as is well known to those skilled in the art. Furthermore, it is evident that said effective daily amount may be lowered or increased depending on the response of the treated
25 subject and/or depending on the evaluation of the physician prescribing the compounds of the instant invention. The effective daily amount ranges mentioned hereinabove are therefore only guidelines.

According to one embodiment, the HCV NS3/4a protease inhibitor of formula (I) and
30 ritonavir may be co-administered once or twice a day, preferably orally, wherein the amount of the compounds of formula (I) per dose is from about 1 to about 2500 mg, and the amount of ritonavir per dose is from 1 to about 2500 mg. In another embodiment, the amounts per dose for once or twice daily co-administration are from about 50 to about 1500 mg of the compound of formula (I) and from about 50 to about
35 1500 mg of ritonavir. In still another embodiment, the amounts per dose for once or twice daily co-administration are from about 100 to about 1000 mg of the compound of formula (I) and from about 100 to about 800 mg of ritonavir. In yet another embodiment, the amounts per dose for once or twice daily co-administration are from

about 150 to about 800 mg of the compound of formula (I) and from about 100 to about 600 mg of ritonavir. In yet another embodiment, the amounts per dose for once or twice daily co-administration are from about 200 to about 600 mg of the compound of formula (I) and from about 100 to about 400 mg of ritonavir. In yet another
5 embodiment, the amounts per dose for once or twice daily co-administration are from about 200 to about 600 mg of the compound of formula (I) and from about 20 to about 300 mg of ritonavir. In yet another embodiment, the amounts per dose for once or twice daily co-administration are from about 100 to about 400 mg of the compound of formula (I) and from about 40 to about 100 mg of ritonavir.

10

Exemplary combinations of the compound of formula (I) (mg)/ritonavir (mg) for once or twice daily dosage include 50/100, 100/100, 150/100, 200/100, 250/100, 300/100, 350/100, 400/100, 450/100, 50/133, 100/133, 150/133, 200/133, 250/133, 300/133, 50/150, 100/150, 150/150, 200/150, 250/150, 50/200, 100/200, 150/200, 200/200,
15 250/200, 300/200, 50/300, 80/300, 150/300, 200/300, 250/300, 300/300, 200/600, 400/600, 600/600, 800/600, 1000/600, 200/666, 400/666, 600/666, 800/666, 1000/666, 1200/666, 200/800, 400/800, 600/800, 800/800, 1000/800, 1200/800, 200/1200, 400/1200, 600/1200, 800/1200, 1000/1200, and 1200/1200. Other exemplary combinations of the compound of formula (I) (mg)/ritonavir (mg) for once or twice
20 daily dosage include 1200/400, 800/400, 600/400, 400/200, 600/200, 600/100, 500/100, 400/50, 300/50, and 200/50.

20

In one embodiment of the present invention there is provided an article of manufacture comprising a composition effective to treat an HCV infection or to inhibit the NS3
25 protease of HCV; and packaging material comprising a label which indicates that the composition can be used to treat infection by the hepatitis C virus; wherein the composition comprises a compound of the formula (I) or any subgroup thereof, or the combination as described herein.

25

Another embodiment of the present invention concerns a kit or container comprising a compound of the formula (I) or any subgroup thereof, or a combination according to the invention combining an HCV NS3/4a protease inhibitor of formula (I) or a pharmaceutically acceptable salt thereof, and ritonavir or a pharmaceutically acceptable salt thereof, in an amount effective for use as a standard or reagent in a test or assay for
30 determining the ability of potential pharmaceuticals to inhibit HCV NS3/4a protease, HCV growth, or both. This aspect of the invention may find its use in pharmaceutical research programs.

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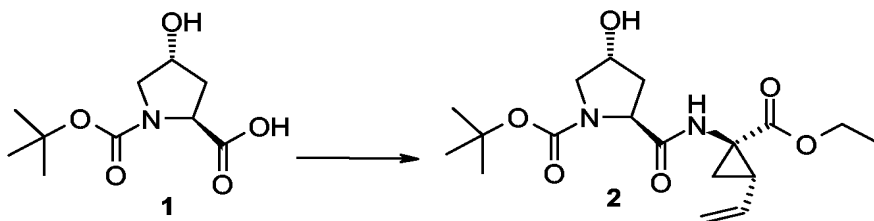
The compounds and combinations of the present invention can be used in high-throughput target-analyte assays such as those for measuring the efficacy of said combination in HCV treatment.

5 Examples

The following examples are intended to illustrate the present invention and not to limit it thereto.

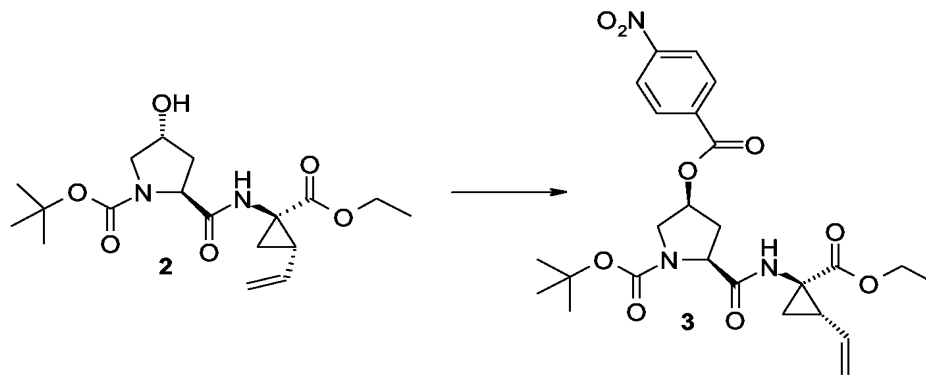
Example 1: Preparation of 17-(5-phenyltetrazol-2-yl)-13-methyl-2,14-dioxo-
10 3,13,15-triazatricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (**9**).

Step A



Boc-protected hydroxyproline **1** (4.0 g, 17.3 mmol), HATU (6.9 g, 18.2 mmol) and the
15 1-(amino)-2-(vinyl)cyclopropanecarboxylic acid ethyl ester (3.5 g, 18.3 mmol) were
dissolved in DMF (60 mL) and cooled to 0°C on ice-bath. Then, DIPEA (6.0 mL) was
added. The ice-bath was removed and the mixture was left at ambient temperature for
12 h. Dichloromethane was then added and the organic phase was washed with aqueous
sodium hydrogen carbonate, citric acid, water, brine and dried (Na₂SO₄). Purification
20 by flash chromatography (gradient ether to ether/methanol, 93:7) gave 6.13 g (96%) of
the target product **2** as an oil.

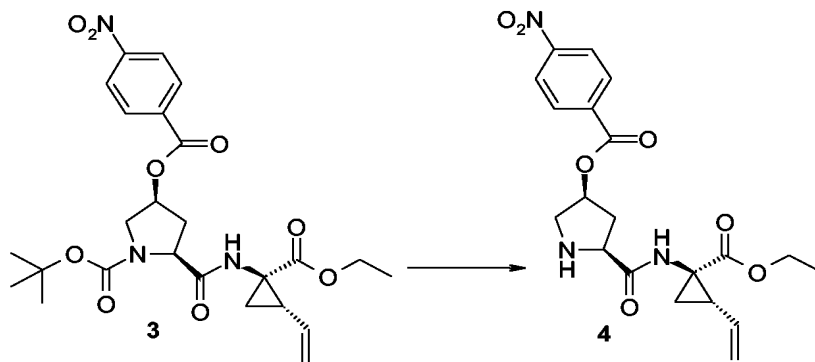
Step B



25 Compound **2** (6.13 g, 16.6 mmol), 4-nitrobenzoic acid (4.17g, 25 mmol) and PPh₃
(6.55 g, 25 mmol) was dissolved in THF (130 mL). The solution was cooled to 0°C and

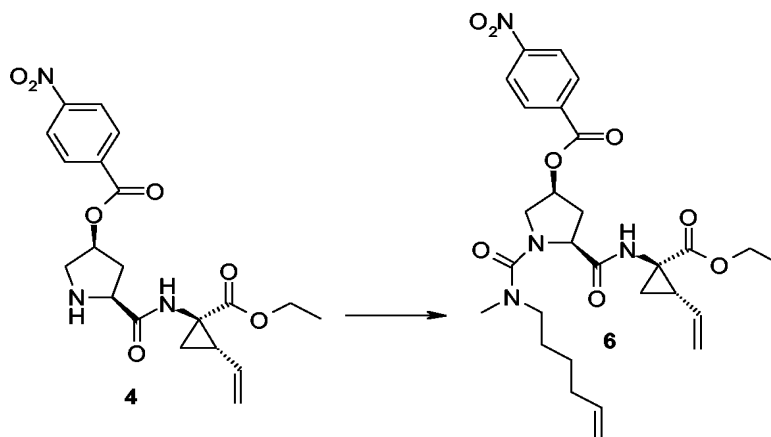
DIAD (5.1 g, 25 mmol) was added slowly. The cooling was then removed and the mixture was left at ambient condition for 12 h. Aqueous sodium hydrogen carbonate (60 mL) was added and the mixture was extracted with dichloromethane, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (gradient pentane/ether, 2:1 to pentane/ether/methanol, 30:68:2) provided 6.2 g (72%) of the desired product **3**: $m/z = 518$ ($\text{M}+\text{H}$)⁺.

Step C



Compound **3** (6.2 g, 12 mmol) was dissolved in an ice-cold mixture of trifluoroacetic acid 33 % in dichloromethane. The ice-bath was then removed and the mixture was left at room temperature for 1.5 h. The solvent was evaporated, and then the residue was partitioned between 0.25 M sodium carbonate and dichloromethane, dried (Na_2SO_4) and evaporated to give 4.8 g (95 %) of the target product **4** as a yellowish powder: $m/z = 418$ ($\text{M}+\text{H}$)⁺.

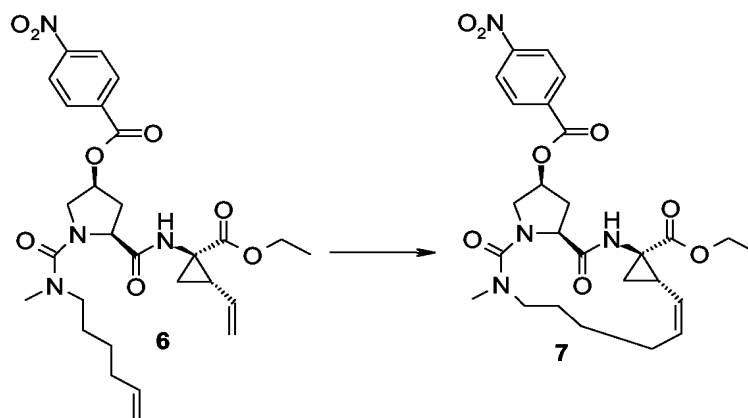
Step D



A tablespoon of sodium hydrogen carbonate was added to a solution of **4** (4.5 g, 10.8 mmol) in THF (160 mL). Then, phosgene (11.3 mL, 20% in toluene) was added. The mixture was stirred vigorously for 1h, then filtered and re-dissolved in dichloro-

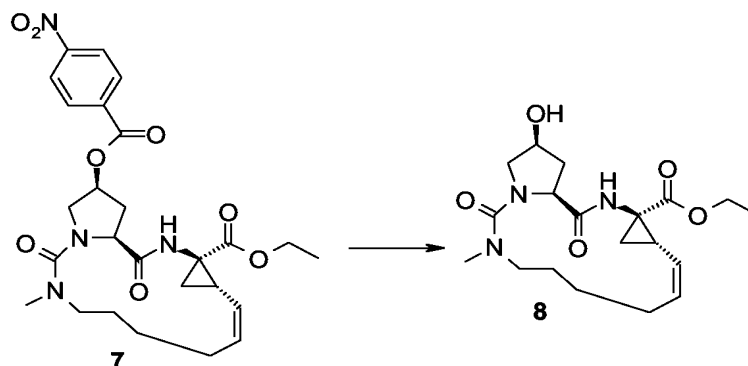
methane (160 mL). Sodium hydrogen carbonate (a tablespoon) was added followed by the *N*-methyl-*N*-hexen-5-yl hydrochloride (2.9 g, 21.6 mmol). The resulting reaction mixture was stirred at room temperature overnight. Workup and purification by flash chromatography (gradient ether to ether/methanol 97:3) gave 5.48g (91%) of the target product **6**: $m/z = 557$ (M+H)⁺.

Step E



Compound **6** (850 mg, 1.53 mmol) was dissolved in 1.5 L of degassed and dried 1,2-dichloroethane and refluxed under argon atmosphere for 12h. Scavenger (MP-TMT, P/N 800470 from Argonaut technologies, ½ teaspoon) was added and the mixture was stirred for 2h, filtered and concentrated by reduced pressure. The crude product was crystallized from dichloromethane/n-hexane to yield 600mg (74%) of the target product **7**: $m/z = 529$ (M+H)⁺.

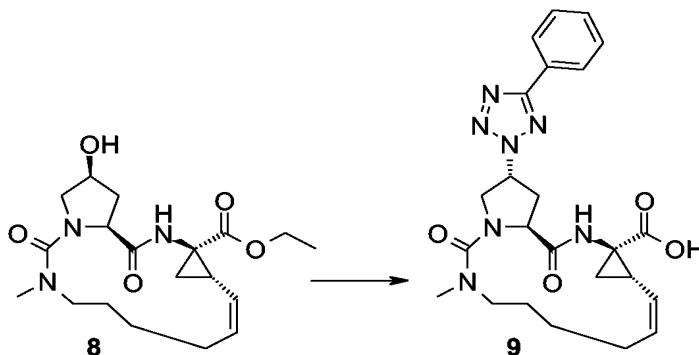
Step F



Compound **7** (200mg, 0.38 mmol) was dissolved in a mixture of methanol/THF/water, 1:2:1, (20 mL) and cooled on ice-bath. Then, lithium hydroxide (1.9 mL, 1 M in water) was added slowly. The mixture was stirred for 4h at 0°C., then neutralized with aqueous acetic acid (20 mL) and extracted with dichloromethane. The organic phase

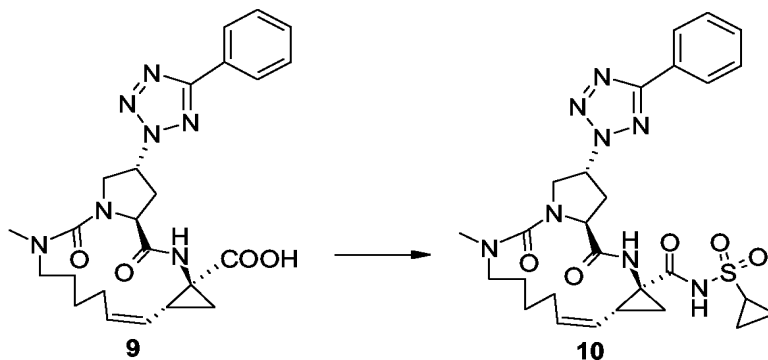
was washed with sodium bicarbonate, water, brine and dried (MgSO_4). Purification by column chromatography (methanol/dichloromethane, 96:4) gave 120 mg of the target compound **8** as a grayish powder: $m/z = 380$ ($\text{M}+\text{H}$)⁺.

5 Step G



DIAD (218 μL , 1.11 mmol) is added at 0°C under nitrogen atmosphere to a solution of **8** (280 mg, 0.738 mmol), 5-phenyl-1H-tetrazole (150 mg, 1.03 mmol) and triphenylphosphine (271 mg, 1.03 mmol) in dry THF (15 mL). Then, the reaction is warmed up to room temperature. After 1.5 h, the solvent is evaporated and the crude product is purified by flash column chromatography (AcOEt/Heptane, 3:7). This product is re-dissolved in THF (15 mL) and MeOH (10 mL). Then, a solution of LiOH (200 mg) in water (3 mL) is added. After 48h, solvent is evaporated and the residue partitioned between water and ether. Aqueous layer is acidified ($\text{pH} = 3$) and extracted with AcOEt, dried (MgSO_4) and evaporated. The residue is crystallized from ether to give the target compound **9**.

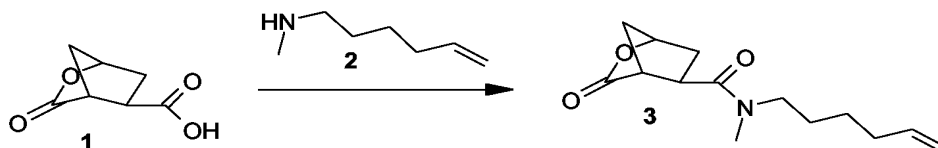
Example 2: Preparation of *N*-[17-(5-phenyltetrazol-2-yl)-13-methyl-2,14-dioxo-3,13,15-triazatricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carbonyl](cyclopropyl)sulfonamide (**10**).



A mixture of **9** (50 mg, 0.104 mmol) and CDI (68 mg, 0.418 mmol) in dry THF (5 mL) is heated at reflux for 2h under nitrogen. The reaction mixture is cooled to room temperature and cyclopropylsulfonamide (101 mg, 0.836 mmol) is added. Then, DBU (73 mg, 0.481 mmol) is added and the reaction mixture is stirred at room temperature for 1h, and then heated at 55°C for 24h. Solvent is evaporated, and the residue partitioned between AcOEt and acidic water (pH = 3). The crude material is purified by column chromatography (AcOEt/CH₂Cl₂/Petroleum ether, 1:1:1). The residue is crystallized in Et₂O, filtered to give the target compound contaminated with the cyclopropylsulfonamide. This material is triturated in 1.5 mL of water, filtered, washed with water and dried overnight in the high vacuum pump to give the target compound ..

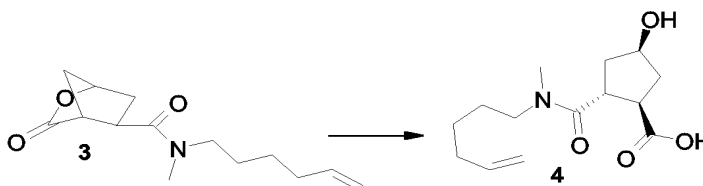
Example 3: Preparation of 17-(5-phenyltetrazol-2-yl)-13-methyl-2,14-dioxo-3,13-diazatricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (**9**).

15 Step A



3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid (**1**, 500 mg, 3.2 mmol) in 4 mL DMF was added at 0°C to HATU (1.34 g, 3.52 mmol) and *N*-methylhex-5-en-1-amine (435 mg, 3.84 mmol) in DMF (3 mL), followed by DIEA. After stirring for 40 min at 0°C, the mixture was stirred at room temperature for 5 h. Then, the solvent was evaporated, the residue dissolved in EtOAc (70 mL) and washed with saturated NaHCO₃ (10 mL). The aqueous phase was extracted with EtOAc (2 x 25 mL). The organic phases were combined, washed with saturated NaCl (20 mL), dried (Na₂SO₄), and evaporated. Purification by flash chromatography (EtOAc/petroleum ether, 2:1) afforded 550 mg (68%) of the target product as a colorless oil: *m/z* = 252 (M+H)⁺.

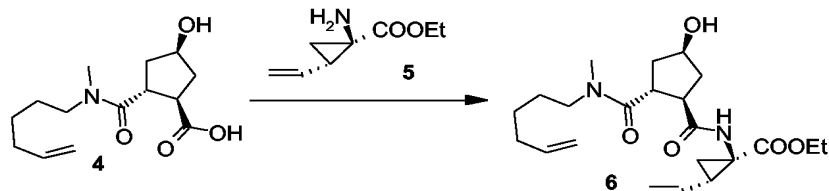
Step B



A solution of LiOH (105 mg in 4 mL of water) was added at 0°C to the lactone amide **3**. After 1h, the conversion was completed (HPLC). The mixture was acidified to pH 2 - 3 with 1N HCl, extracted with AcOEt, dried (MgSO₄), evaporated, co-evaporated

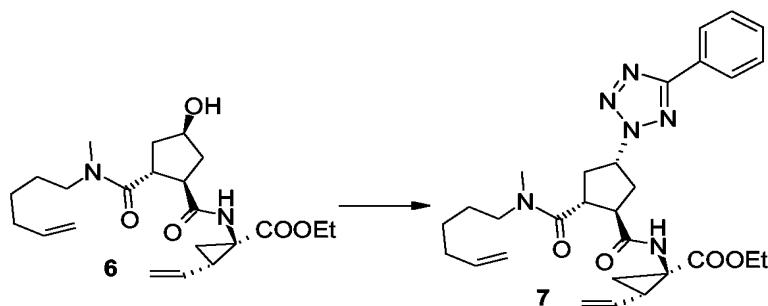
with toluene several times, and dried under high vacuum overnight to give 520 mg (88%) of the target product: $m/z = 270$ ($M+H$)⁺.

Step C



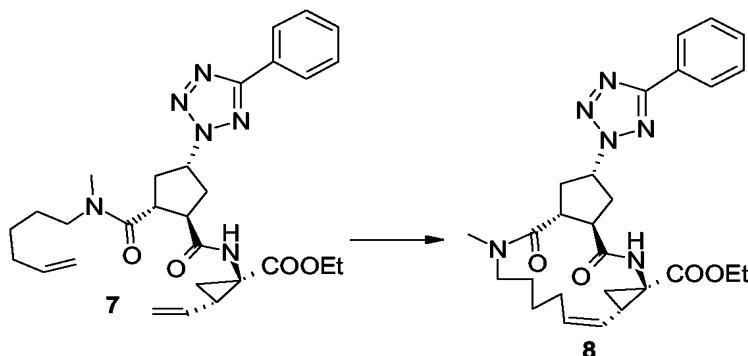
The 1-(amino)-2-(vinyl)cyclopropanecarboxylic acid ethyl ester hydrochloride (4.92 g, 31.7 mmol) and HATU (12.6 g, 33.2 mmol) were added to 4 (8.14 g, 30.2 mmol). The mixture was cooled in an ice bath under argon, and then DMF (100 mL) and DIPEA (12.5 mL, 11.5 mmol) were successively added. After 30 min at 0°C, the solution was stirred at room temperature for an additional 3 h. Then, the reaction mixture was partitioned between EtOAc and water, washed successively with 0.5 N HCl (20 mL) and saturated NaCl (2 x 20 mL), and dried (Na₂SO₄). Purification by flash chromatography (AcOEt/CH₂Cl₂/Petroleum ether, 1:1:1) afforded 7.41 g (60%) of the target product 6 as a colorless oil: $m/z = 407$ ($M+H$)⁺.

Step D



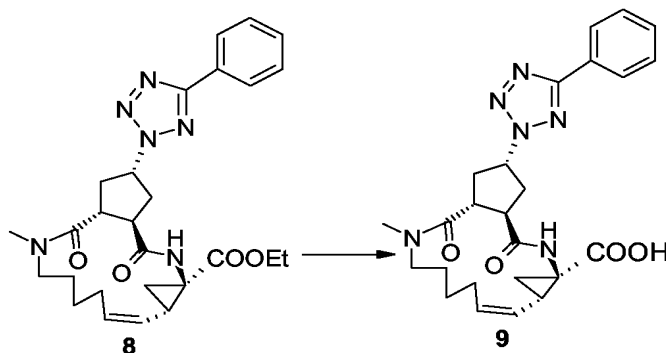
DIAD (218 μ L, 1.11 mmol) was added at 0°C under nitrogen atmosphere to a solution of 6 (300 mg, 0.738 mmol), 5-phenyl-1H-tetrazole (150 mg, 1.03 mmol) and triphenylphosphine (271 mg, 1.03 mmol) in dry THF (15 mL). Then, the reaction was warmed up to room temperature. After 1.5 h, the solvent was evaporated and the crude product was purified by flash column chromatography (AcOEt/Heptane, 3:7) to give 142 mg (43%) of the target product 7: $m/z = 535$ ($M+H$)⁺.

Step E



A solution of **7** (175 mg, 0.33 mmol) and Hoveyda-Grubbs 1st generation catalyst (39 mg, 0.066 mmol) in dried and degassed 1,2-dichloroethane (200 mL) was heated at 70°C under nitrogen for 12 h. Then, the solvent was evaporated and the residue purified by silica gel chromatography (Petroleum ether/CH₂Cl₂/Et₂O; 3:1:1) to give 83 mg (50%) of the target product: $m/z = 507$ (M+H)⁺.

Step F

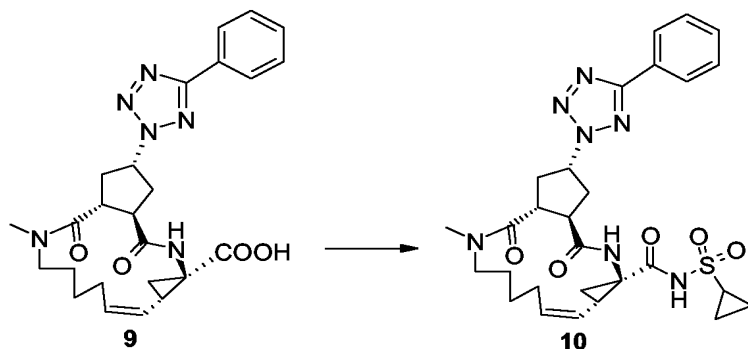


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A solution of LiOH (200 mg) in water (3 mL) was added to a stirred solution of **8** in THF (15 mL) and MeOH (10 mL). After 48h, solvent was evaporated and the residue partitioned between water and ether. Aqueous layer was acidified (pH = 3) and extracted with AcOEt, dried (MgSO₄) and evaporated. The residue was crystallized from ether to give 68 mg (87%) of the target compound **9**: $m/z = 479$ (M+H)⁺.

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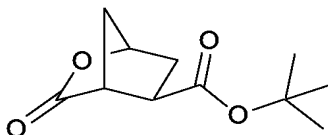
Example 4: Preparation of *N*-[17-(5-phenyltetrazol-2-yl)-13-methyl-2,14-dioxo-3,13-diazatricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carbonyl](cyclopropyl)sulfonamide (**10**).



A mixture of **9** (50 mg, 0.104 mmol) and CDI (68 mg, 0.418 mmol) in dry THF (5 mL) was heated at reflux for 2h under nitrogen. The reaction mixture was cooled to room temperature and cyclopropylsulfonamide (101 mg, 0.836 mmol) was added. Then, DBU (73 mg, 0.481 mmol) was added and the reaction mixture was stirred at room temperature for 1h, and then heated at 55°C for 24h. Solvent was evaporated, and the residue partitioned between AcOEt and acidic water (pH = 3). The crude material was purified by column chromatography (AcOEt/CH₂Cl₂/Petroleum ether, 1:1:1). The residue was crystallized in Et₂O, filtered to give the target compound contaminated with the cyclopropylsulfonamide. This material was triturated in 1.5 mL of water, filtered, washed with water and dried overnight in the high vacuum pump to give 28 mg (46%) of the target compound as a white powder: $m/z = 582$ (M+H)⁺.

Example 5

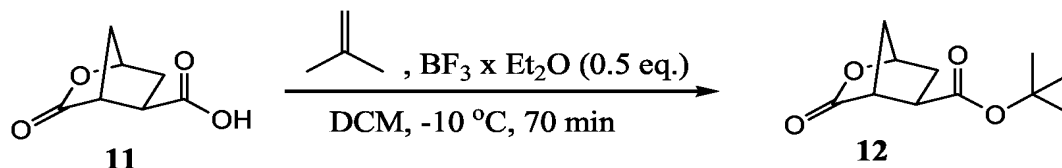
Synthesis of 3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid *tert*-butyl ester (**12**)



DMAP (14 mg, 0.115 mmol) and Boc₂O (252 mg, 1.44 mmol) was added to a stirred solution of **11** (180 mg, 1.15 mmol) in 2 mL CH₂Cl₂ under inert argon atmosphere at 0°C. The reaction was allowed to warm to room temperature and was stirred overnight. The reaction mixture was concentrated and the crude product was purified by flash column chromatography (toluene/ethyl acetate gradient 15:1, 9:1, 6:1, 4:1, 2:1) which gave the title compound (124 mg, 51%) as white crystals.

¹H-NMR (300 MHz, CD₃OD) δ 1.45 (s, 9H), 1.90 (d, $J = 11.0$ Hz, 1H), 2.10-2.19 (m, 3H), 2.76-2.83 (m, 1H), 3.10 (s, 1H), 4.99 (s, 1H); ¹³C-NMR (75.5 MHz, CD₃OD) δ 27.1, 33.0, 37.7, 40.8, 46.1, 81.1, 81.6, 172.0, 177.7.

Alternative method for the preparation of compound **12**



Compound **11** (13.9 g, 89 mmol) was dissolved in dichloromethane (200 ml) and then cooled to approximately -10°C under nitrogen. Isobutylene was then bubbled into the solution until the total volume had increased to approximately 250 ml which gave a “cloudy solution”. $\text{BF}_3 \times \text{Et}_2\text{O}$ (5.6 ml, 44.5 mmol, 0.5 eq.) was added and the reaction mixture was kept at approximately -10°C under nitrogen. After 10 min, a clear solution was obtained. The reaction was monitored by TLC (EtOAc-Toluene 3:2 acidified with a few drops of acetic acid and hexane-EtOAc 4:1, staining with basic permanganate solution). At 70 min only traces of compound **11** remained and aq. saturated NaHCO_3 (200 ml) was added to the reaction mixture, which was then stirred vigorously for 10 min. The organic layer was washed with saturated NaHCO_3 (3 x 200 ml) and brine (1 x 150 ml), then dried with sodium sulfite, filtered and concentrated into an oil containing small droplets. Upon addition of hexane to the residue the product crashed out. Addition of more hexane and heating to reflux gave a clear solution from which the product crystallized. The crystals were collected by filtration and was washed with hexane (rt), then air-dried for 72 h giving colourless needles (12.45 g, 58.7 mmol, 66% from first harvest).

Example 6: Activity of compounds of formula (I)

Replicon assay

The compounds of formula (I) were examined for activity in the inhibition of HCV RNA replication in a cellular assay. The assay demonstrated that the compounds of formula (I) exhibited activity against HCV replicons functional in a cell culture. The cellular assay was based on a bicistronic expression construct, as described by Lohmann et al. (1999) Science vol. 285 pp. 110-113 with modifications described by Krieger et al. (2001) Journal of Virology 75: 4614-4624, in a multi-target screening strategy. In essence, the method was as follows.

The assay utilized the stably transfected cell line Huh-7 luc/neo (hereafter referred to as Huh-Luc). This cell line harbors an RNA encoding a bicistronic expression construct comprising the wild type NS3-NS5B regions of HCV type 1b translated from an Internal Ribosome Entry Site (IRES) from encephalomyocarditis virus (EMCV), preceded by a reporter portion (FfL-luciferase), and a selectable marker portion (neo^R , neomycine phosphotransferase). The construct is bordered by 5' and 3' NTRs (non-

translated regions) from HCV type 1b. Continued culture of the replicon cells in the presence of G418 (neo^R) is dependent on the replication of the HCV RNA. The stably transfected replicon cells that express HCV RNA, which replicates autonomously and to high levels, encoding *inter alia* luciferase, are used for screening the antiviral

5 compounds.

The replicon cells were plated in 384 well plates in the presence of the test and control compounds which were added in various concentrations. Following an incubation of three days, HCV replication was measured by assaying luciferase activity (using
10 standard luciferase assay substrates and reagents and a Perkin Elmer ViewLux[™] ultraHTS microplate imager). Replicon cells in the control cultures have high luciferase expression in the absence of any inhibitor. The inhibitory activity of the compound on luciferase activity was monitored on the Huh-Luc cells, enabling a dose-response curve for each test compound. EC50 values were then calculated, which
15 value represents the amount of the compound required to decrease by 50% the level of detected luciferase activity, or more specifically, the ability of the genetically linked HCV replicon RNA to replicate.

Inhibition assay

20 The aim of this *in vitro* assay was to measure the inhibition of HCV NS3/4A protease complexes by the compounds of the present invention. This assay provides an indication of how effective compounds of the present invention would be in inhibiting HCV NS3/4A proteolytic activity.

25 The inhibition of full-length hepatitis C NS3 protease enzyme was measured essentially as described in Poliakov, 2002 Prot Expression & Purification 25 363 371. Briefly, the hydrolysis of a depsipeptide substrate, Ac-DED(Edans)EEAbuψ[COO]ASK(Dabcyl)-NH₂ (AnaSpec, San José, USA), was measured spectrofluorometrically in the presence of a peptide cofactor, KKGSVVIVGRIVLSGK (Åke Engström, Department of
30 Medical Biochemistry and Microbiology, Uppsala University, Sweden). [Landro, 1997 #Biochem 36 9340-9348]. The enzyme (1 nM) was incubated in 50 mM HEPES, pH 7.5, 10 mM DTT, 40% glycerol, 0.1% n-octyl-D-glucoside, with 25 µM NS4A cofactor and inhibitor at 30 °C for 10 min, whereupon the reaction was initiated by addition of 0.5 µM substrate. Inhibitors were dissolved in DMSO, sonicated for 30 sec. and
35 vortexed. The solutions were stored at - 20°C between measurements.

The final concentration of DMSO in the assay sample was adjusted to 3.3%. The rate of hydrolysis was corrected for inner filter effects according to published procedures.

[Liu, 1999 Analytical Biochemistry 267 331-335]. K_i values were estimated by non-linear regression analysis (GraFit, Erithacus Software, Staines, MX, UK), using a model for competitive inhibition and a fixed value for K_m (0.15 μM). A minimum of two replicates was performed for all measurements.

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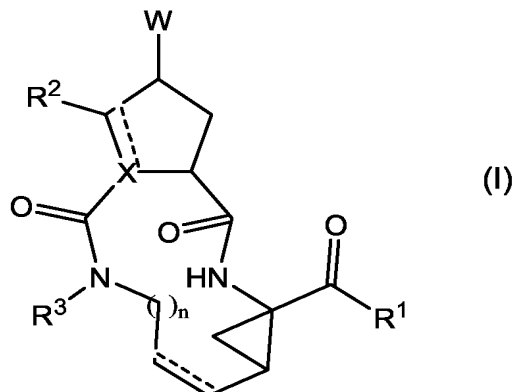
The following Table 1 lists compounds that were prepared according to any one of the above examples. The activities of the compounds tested are also depicted in Table 1.

Compound nr.	Example nr.	EC_{50} (μM)	K_i (nM)
		Replicon assay	Enzymatic assay
9	Example 3	>10	>1000
10	Example 4	3.427	30

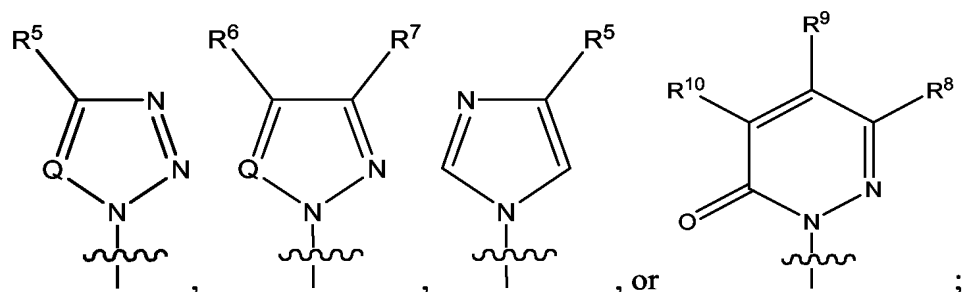
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Claims

1. A compound having the formula

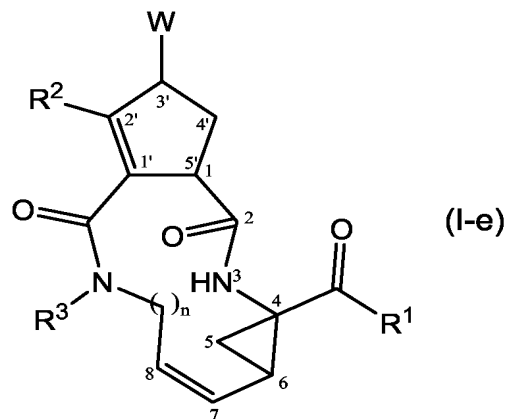
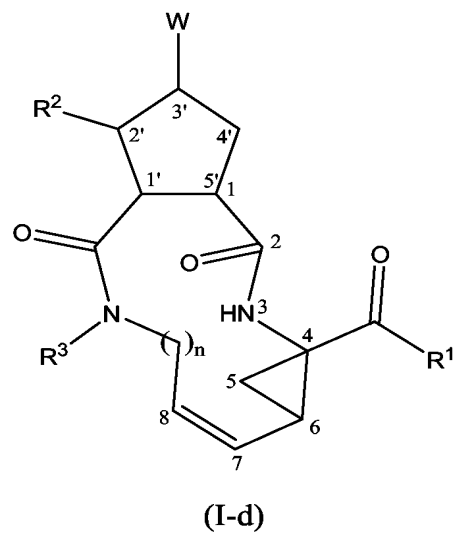
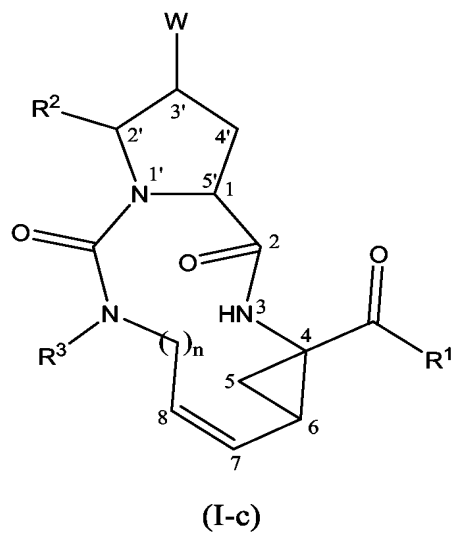


- 5 an *N*-oxide, salt, or stereoisomer thereof,
 wherein each dashed line (represented by - - - -) represents an optional double bond;
X is N, CH and where **X** bears a double bond it is C;
R¹ is -OR¹¹, -NH-SO₂R¹²;
R² is hydrogen, and where **X** is C or CH, **R**² may also be C₁₋₆alkyl;
 10 **R**³ is hydrogen, C₁₋₆alkyl, C₁₋₆alkoxyC₁₋₆alkyl, or C₃₋₇cycloalkyl;
n is 3, 4, 5, or 6;
W is a heterocycle of formula



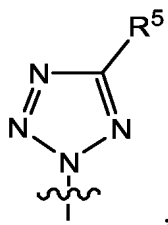
- Q** is N or CR⁴;
 15 **R**⁴ is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
 thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl;
R⁵ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl
 optionally substituted with C₁₋₆alkyl; or pyridyl;
 one of **R**⁶, **R**⁷ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
 20 thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl; while the other of **R**⁶, **R**⁷
 is hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy;
 thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl;

- one of **R**⁸, **R**⁹, **R**¹⁰ is phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl; while the other two of **R**⁸, **R**⁹, **R**¹⁰ independently are hydrogen; phenyl optionally substituted with C₁₋₆alkyl or with C₁₋₆alkoxy; thiazolyl optionally substituted with C₁₋₆alkyl; or pyridyl;
- R**¹¹ is hydrogen; aryl; Het; C₃₋₇cycloalkyl optionally substituted with C₁₋₆alkyl; or C₁₋₆alkyl optionally substituted with C₃₋₇cycloalkyl, aryl or with Het;
- R**¹² is aryl; Het; C₃₋₇cycloalkyl optionally substituted with C₁₋₆alkyl; or C₁₋₆alkyl optionally substituted with C₃₋₇cycloalkyl, aryl or with Het;
- each **aryl** as a group or part of a group is phenyl, optionally substituted with one, two or three substituents selected from halo, hydroxy, nitro, cyano, carboxyl, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkoxyC₁₋₆alkyl, C₁₋₆alkylcarbonyl, amino, mono- or diC₁₋₆alkylamino, azido, mercapto, polyhaloC₁₋₆alkyl, polyhaloC₁₋₆alkoxy, C₃₋₇cycloalkyl, pyrrolidinyl, piperidinyl, piperazinyl, 4-C₁₋₆alkyl-piperazinyl, 4-C₁₋₆alkylcarbonyl-piperazinyl, morpholinyl; wherein the morpholinyl and piperidinyl groups may be optionally substituted with one or with two C₁₋₆alkyl radicals; and
- each **Het** as a group or part of a group is a 5 or 6 membered saturated, partially unsaturated or completely unsaturated heterocyclic ring containing 1 to 4 heteroatoms each independently selected from nitrogen, oxygen and sulfur, and being optionally substituted with one, two or three substituents each independently selected from the group consisting of halo, hydroxy, nitro, cyano, carboxyl, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkoxyC₁₋₆alkyl, C₁₋₆alkylcarbonyl, amino, mono- or diC₁₋₆alkylamino, azido, mercapto, polyhaloC₁₋₆alkyl, polyhaloC₁₋₆alkoxy, C₃₋₇cycloalkyl, pyrrolidinyl, piperidinyl, piperazinyl, 4-C₁₋₆alkyl-piperazinyl, 4-C₁₋₆alkylcarbonyl-piperazinyl, morpholinyl; wherein the morpholinyl and piperidinyl groups may be optionally substituted with one or with two C₁₋₆alkyl radicals.
2. A compound according to claim 1, wherein the compound has the formula (I-c), (I-d), or (I-e):



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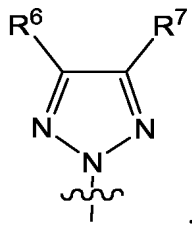
3. A compound according to any one of claims 1-2, wherein W is



and R^5 is phenyl, 3-methoxyphenyl, 3,4-dimethoxyphenyl, 4-ethoxyphenyl, 4-propoxyphenyl, 4-butoxyphenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl.

10

4. A compound according to any one of claims 1-2, wherein W is



wherein R^6 is phenyl, m-methoxyphenyl, 2-pyridyl, 3-pyridyl, or 2-thiazolyl; and R^7 is phenyl, p-methoxyphenyl, or 4-ethoxyphenyl.

5

5. A compound according to any one of claims 1-4, wherein
 (a) R^1 is $-OR^{11}$, wherein R^{11} is C_{1-6} alkyl or hydrogen; or
 (b) R^1 is $-NHS(=O)_2R^{12}$, wherein R^{12} is methyl, cyclopropyl, or phenyl.

- 10 6. A compound according to any of claims 1-5 other than an N-oxide, or salt.

7. A combination comprising
 (a) a compound as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof; and
 15 (b) ritonavir, or a pharmaceutically acceptable salt thereof.

8. A pharmaceutical composition comprising a carrier, and as active ingredient an anti-virally effective amount of a compound as claimed in any one of claims 1-6 or a combination according to claim 7.

20

9. A compound according to any of claims 1-6 or a combination according to claim 7, for use as a medicament.

10. Use of a compound according to any of claims 1-6 or a combination according to claim 7, for the manufacture of a medicament for inhibiting HCV replication.

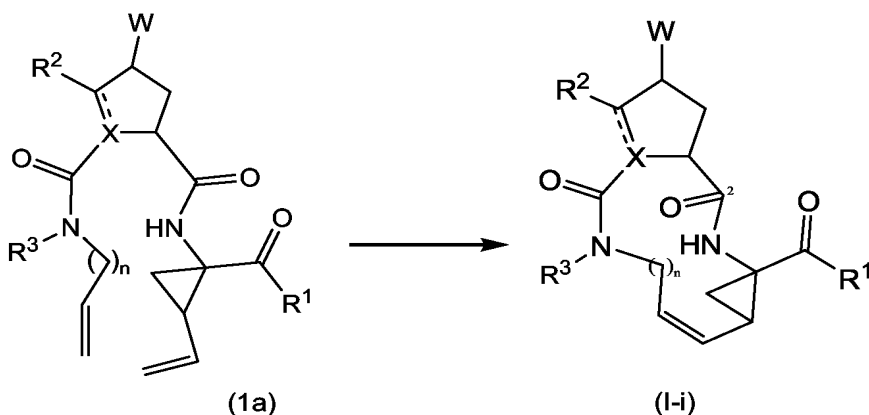
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11. A method of inhibiting HCV replication in a warm-blooded animal said method comprising the administration of an effective amount of a compound according to any of claims 1-6 or an effective amount of each component of the combination according to claim 7.

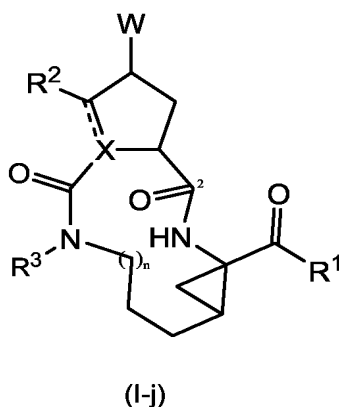
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12. A process for preparing a compound as claimed in any of claims 1 - 6, wherein said process comprises:

- 5 (a) preparing a compound of formula (I) wherein the bond between C₇ and C₈ is a double bond, which is a compound of formula (I-i), by forming a double bond between C₇ and C₈, in particular via an olefin metathesis reaction, with concomitant cyclization to the macrocycle as outlined in the following reaction scheme:

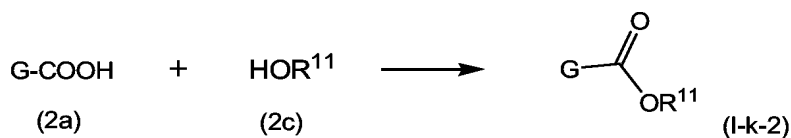
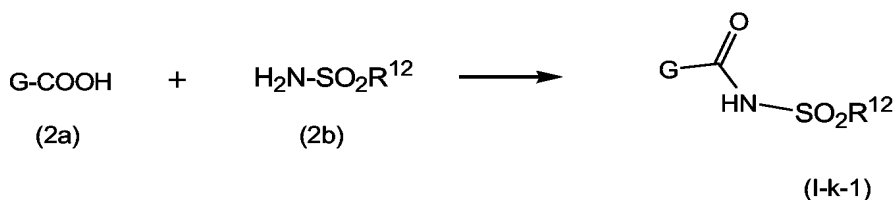
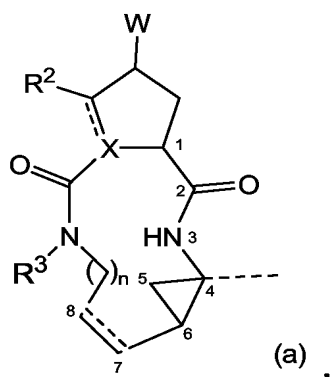


- 10 (b) converting a compound of formula (I-i) to a compound of formula (I) wherein the link between C₇ and C₈ in the macrocycle is a single bond, i.e. a compound of formula (I-j):

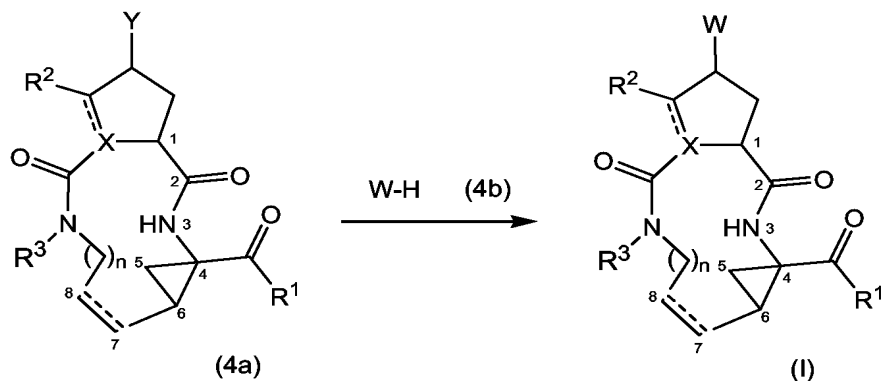


by a reduction of the C₇-C₈ double bond in the compounds of formula (I-i);

- 15 (c) preparing a compound of formula (I) wherein R¹ represents -NHSO₂R¹², said compounds being represented by formula (I-k-1), by forming an amide bond between an intermediate (2a) and a sulfonylamine (2b), or preparing a compound of formula (I) wherein R¹ represents -OR¹¹, i.e. a compound (I-k-2), by forming an ester bond between an intermediate (2a) and an alcohol (2c) as outlined in the following scheme wherein G represents a group:
- 20

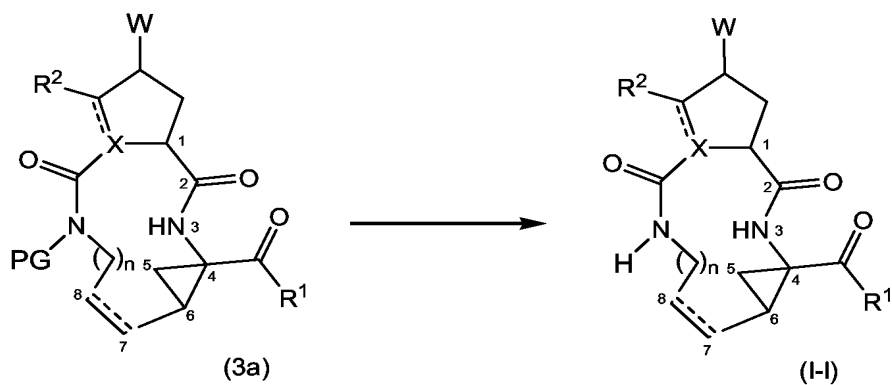


5 (d) reacting an intermediate (4a) with an intermediate (4b) as outlined in the following reaction scheme:



wherein Y in (4a) represents hydroxy or a leaving group; which reaction in particular is an O-arylation reaction wherein Y represents a leaving group, or a Mitsunobu reaction, wherein Y represents hydroxy;

(e) preparing a compound of formula (I) wherein R³ is hydrogen, said compound being represented by (I-l), from a corresponding nitrogen-protected intermediate (3a), wherein PG represents a nitrogen protecting group:



(f) converting compounds of formula (I) into each other by a functional group transformation reaction; or

- 5 (g) preparing a salt form by reacting the free form of a compound of formula (I) with an acid or a base.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/064818

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K5/078 A61K38/05 C07D403/04 A61K31/41 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/072243 A (ENANTA PHARMACEUTICALS, INC; MIAO, ZENWEI; SUN, YING; WU, FRANK; NAKAJ) 26 August 2004 (2004-08-26) cited in the application Abstract; claims; examples, e.g. nos. 1, 27, 32, 41, 47, 55, 71-73.	1-12
P, A	WO 2005/073216 A (MEDIVIR AB; ROSENQUIST, AASA; THORSTENSSON, FREDRIK; JOHANSSON, PER-OL) 11 August 2005 (2005-08-11) Abstract; claims; examples, e.g. nos. 139, 140.	1-12
P, A	WO 2005/073195 A (MEDIVIR AB; ROSENQUIST, AASA; THORSTENSSON, FREDRIK; JOHANSSON, PER-OL) 11 August 2005 (2005-08-11) Abstract; claims; examples, e.g. nos. 110, 111.	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

24 October 2006

Date of mailing of the international search report

31/10/2006

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/064818

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 11 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2006/064818

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004072243 A	26-08-2004	AU 2004211637 A1 CA 2515216 A1 EP 1590442 A2	26-08-2004 26-08-2004 02-11-2005
WO 2005073216 A	11-08-2005	AR 048401 A1 AR 047793 A1 AU 2005207815 A1 AU 2005207816 A1 CA 2552317 A1 CA 2552319 A1 WO 2005073195 A2	26-04-2006 22-02-2006 11-08-2005 11-08-2005 11-08-2005 11-08-2005 11-08-2005
WO 2005073195 A	11-08-2005	AR 048401 A1 AR 047793 A1 AU 2005207815 A1 AU 2005207816 A1 CA 2552317 A1 CA 2552319 A1 WO 2005073216 A2	26-04-2006 22-02-2006 11-08-2005 11-08-2005 11-08-2005 11-08-2005 11-08-2005